NOTICE OF GRANT AWARDS REPORT

RESEARCH
Department of Health and Human Services
National Institutes of Health

NATIONAL INSTITUTE OF NEUROLOGICAL DISORDERS AND STROKE

Grant Number: 2 R01 NS035103-08A1
Principal Investigator: KRUBITZER, LEAH A PHD
Project Title: Somatosensory Cortex and Thalamus

VICE CHANCELLOR FOR RESEARCH
UNIVERSITY OF CALIFORNIA, DAVIS
OVC, SPON PGMS
ONE SHIELDS AVENUE
DAVIS, CA 95616-671
UNITED STATES
Award e-mailed to: vcresearch@ucdavis.edu

Budget Period: 04/01/2005 - 01/31/2006
Project Period: 05/01/1997 - 01/31/2010

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $326,378 (see "Award Calculation" in Section I) to Regents of the University of California in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to terms and conditions referenced below.

Acceptance of this award including the Terms and Conditions is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Award recipients are responsible for reporting inventions derived or reduced to practice in the performance of work under this grant. Rights to inventions vest with the grantee organization provided certain requirements are met and there is acknowledgement of NIH support. In addition, recipients must ensure that patent and license activities are consistent with their responsibility to make unique research resources developed under this award available to the scientific community, in accordance with NIH policy. For additional information, please visit http://www.iedison.gov.

If you have any questions about this award, please contact the individual(s) referenced in the information below.

Page No. 1
Sincerely yours,

King P. Bond, Jr.
Grants Management Officer
NATIONAL INSTITUTE OF NEUROLOGICAL DISORDERS AND STROKE

See additional information below

SECTION I - AWARD DATA - 2 R01 NS035103-08A1

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<tr>
<th>AWARD CALCULATION (U.S. Dollars):</th>
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Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project, is as follows.
09 $319,796
10 $320,442
11 $320,656
12 $320,656

FISCAL INFORMATION:
CFDA 93.853
Number: [Redacted]
EIN: [Redacted]
Document Number: RNS035103C

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NIH ADMINISTRATIVE DATA:
PCC: EDWARECN / OC: 41.4B /Processed: BONDKI 050131 0242

SECTION II - PAYMENT/HOTLINE INFORMATION - 2 R01 NS035103-08A1

For Payment and HHS Office of Inspector General Hotline Information, see the NIH Home Page at
SECTION III - TERMS AND CONDITIONS - 2 R01 NS035103-08A1

This award is based on the application submitted to, and as approved by, the NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Grant Award.
b. The restrictions on the expenditure of federal funds in appropriations acts, to the extent those restrictions are pertinent to the award.
c. 45 CFR Part 74 or 45 CFR Part 92 as applicable.
d. The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(see NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

This grant is awarded under the terms and conditions of the Federal Demonstration Partnership Phase IV.

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

Treatment of Program Income:
Additional Costs

SECTION IV - NINDS SPECIAL TERMS AND CONDITIONS

This grant has been selected under the NINDS plan to redistribute grant workload more evenly throughout the year. Consequently, the budget period reflects a January 31st end date. Subsequent budget periods will begin on February 1, and will be for a 12-month duration. Although this grant will have a slightly shorter budget period this year, it is awarded the full 12-month level of funds for the budget period. Additional time may be requested at the end of the project period, if needed.

In order to meet Institute program objectives within Fiscal Year 2005
NOTICE OF GRANT AWARDS REPORT

budget constraints, this grant is reduced to a level below that recommended. Future year levels of support are determined by applying an administrative reduction.

This is a modular grant award without direct cost categorical breakdown in accordance with the guidelines published in the NIH Grants Policy Statement (http://grants1.nih.gov/grants/policy/nihgps_2003/index.htm). Recipients are required to allocate and account for costs related to this award by category within their institutional accounting system in accordance with applicable cost principles.

The program official is responsible for the scientific, programmatic and technical aspects of this project. The grants management specialist is responsible for the negotiation, award and administration of this project and for interpretation of grants administration policies and provisions. These individuals work together in overall project administration. Prior approval requests (countersigned by the PI & authorized business official) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail provided they are routed through these same officials (listed below.) For additional information, you may access the NIH home page at http://www.nih.gov/ and the NINDS Home Page at http://www.ninds.nih.gov.

EMMELINE EDWARDS, Program Official
Phone: 301-496-9964 Email: ee48r@nih.gov Fax: 301-402-2060

Pamela L Mayer, Grants Specialist
Phone: 301-496-4207 Email: mayerp@mail.nih.gov Fax: 301-451-5635

SPREADSHEET
GRANT NUMBER: 2 R01 NS035103-08A1

P.I.: KRUBITZER, LEAH A
INSTITUTION: Regents of the University of California

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**Title of Project:** Somatosensory Cortex and Thalamus

**Principal Investigator/Program Director:**

**Name:** Krubitzer, Leah Ann

**Position Title:** Professor, II

**Department, Service, Laboratory, or Equivalent:** Center for Neuroscience

**Major Subdivision:** Division of Biological Sciences

**Telephone and Fax:**

**E-mail Address:**

**Human Subjects Research:**

**Research Exempt:** Yes

**Human Subjects Assurance No.:**

**NIH-defined Phase III Clinical Trial:**

**Mailing Address:**

**Street, City, State, Zip Code:**

**Center for Neuroscience**

**University of California, Davis**

**Personal Info:**

**Davis, CA 95616**

**Dates of Proposed Period of Support:**

**From:** 4/1/05

**Through:** 3/31/10

**Costs Requested for Initial Budget Period:**

**Direct Costs ($):** $250,000

**Total Costs ($):** $369,589

**Costs Requested for Proposed Period of Support:**

**Direct Costs ($):** $1,250,000

**Total Costs ($):** $1,869,686

**Applicant Organization:**

**Name:** The Regents of the University of California

**Address:** OVCH, Sponsored Programs

**One Shields Avenue**

**University of California**

**Davis, CA 95616-8671**

**Institutional Profile File Number (if known):** 577503

**Administrative Official to be Notified if Award is Made:**

**Name:** Vice Chancellor for Research

**Address:** OVCH, Sponsored Programs,

**One Shields Avenue**

**University of California**

**Davis, CA 95616-8671**

**Principal Investigator/Program Director Assurance:**

**Signature of PI/PD Named in 3a.**

**Date:** 6/10/04

**Signature of Official Named in 13.**

**Date:** JUN 14, 2004
While area 5 has been considered a posterior parietal field involved exclusively in processing somatic inputs, recent evidence from our laboratory in both New World and Old World monkeys, as well as work from other laboratories, indicate that this cortical area is also involved in processing visual inputs, and is closely associated with the motor system. Accumulating evidence indicates that area 5 may be a “central planner” critical for monitoring limb location during intended reaching and grasping, converting sensory locations into motor coordinates for intentional movement, and in perceiving the movements of the body in extra personal space. The goal of the present investigation is to determine the role of posterior parietal area 5 in visually guided and non-Visually guided reaching and grasping, object manipulation, bilateral coordination of the hands, and information transfer across the cerebral hemispheres. To accomplish this, we will make electrophysiologically targeted unilateral lesions in the hand and forearm representation of area 5 in macaque monkeys, and examine the effects of these lesions on these behaviors. We expect that ablation of area 5 will result in a variety of deficits involving manual dexterity, reaching, grasping, and bilateral coordination of the hands. The proposed studies are broken into three major groups of experiments. The first series of experiments will examine the cortical, callosal, and subcortical connections of area 5 and adjacent somatosensory area 2, in macaque monkeys. The second group of experiments will examine the consequences of precisely targeted lesions in area 5 on directed reaching and grasping, bilateral coordination of the hands, shape discrimination abilities and interhemispheric transfer. The tasks include reaching and grasping under visually guided and non-Visually guided conditions, bilateral manipulation of objects, and object identification under both ipsilateral and bilateral hand use conditions. The final series of experiments will examine the cortical substrate for behavioral recovery by determining if changes in both functional organization and anatomical cortical connectivity have occurred in cortical area 2 as a consequence of the lesion. This study represents one of the first attempts to combine modern neuroanatomical, electrophysiological, and lesioning techniques to determine the contribution of a single cortical field involved in generating sophisticated hand use. Further, it is one of the few studies that utilizes electrophysiological and neuroanatomical techniques to examine the long-term cortical changes that occur after cortical damage, followed by behavioral training. These studies will ultimately allow us to better understand the role of area 5 in reaching, grasping, object manipulation, and bilateral coordination of the hands, the time course of behavioral plasticity following lesions in area 5, and the cortical mechanisms that contribute to recovery after brain injury.
# RESEARCH GRANT

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| Appendix (Five called sets. No page numbering necessary for Appendix.)               | 25 |
| Number of publications and manuscripts accepted or submitted for publication (not to exceed 10) | 26 |
| Other items (list): 3                                                               | 27 |

## Appendix

3. Personal Info
4. Personal Info
5. Personal Info
MODULAR BUDGET – YEARS 1 – 5.

<table>
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<th>Year 1</th>
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BUDGET JUSTIFICATION:

**Personnel: ($192,430)**

**Principal Investigator: Leah Krubitzer**

The PI will play a large role in electrophysiological recording experiments, lesioning experiments, and anatomical tracing experiments. She will oversee the experimental design and data analysis. In addition, she will be responsible for manuscript preparation.
Other Expenses: ($8407)

Supplies: ($31,000 year 1, $25,000 years 2-5)

2 primate containment housing, 2LCH-3 @ $2,250 x 2 = $4,500

2 Sony Digital 8 Handycam + accessories (e.g. remote control tripods, battery charger, memory sticks) = $1,600

Histological Supplies: This includes chemicals for reactions such as cytochrome oxidase, myelin stains, biotinylated dextran amine (BDA), immunohistochemistry, general chemicals (e.g., alcohol, xylene), and fixatives. Glassware, slides, coverslips, microtome maintenance, and slide boxes are also necessary. Also included in this cost is the disposal of hazardous waste. The University requires each laboratory to collect all biohazardous waste products, dispose of, and remove waste according to EH&S requirements. We are charged for containers as well as waste removal.

Anatomical tracers: Fluorescent tracers (fluoroemerald, fluororuby) and BDA will be used in these studies.

Surgery costs: This includes the cost of using the sterile surgical facilities at the primate center. This also includes pharmaceuticals such as isoflurane, ketamine, xylolcaine, and atropine, as well as disposable items such as gowns, booties, gloves, caps, swabs, gauze, syringes, lactated ringers, gel foam, bone wax, and surgical.

Computer and Microscope Supplies: This includes CD’s, removable hard drives for 3D reconstructions, printer hardware, plotter hardware, plotter inks, and fluorescent bulbs for microscopes.

Travel: $1,500

Round trip airfares plus accommodation and per diem expenses for the principal investigator to attend the Society for Neuroscience meeting each year.

Animal costs: ($23,400 year 1, $11,300 years 2-5)

Animal: 8 monkeys year 1 @ $2,200 = $17,600
10 monkeys in years 2-5 = $22,000/4 = $5,500 per year

Per dium: 9 monkeys @ $4.42 x 2 years each = $29,000 over 5 years = $5,800/per year
Years 2 – 5
A 4% increase per annum has been allowed for all amounts in the budget projections for years two through five.
BIOPHYSICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow the sample format for each person. DO NOT EXCEED FOUR PAGES

NAME
Leah Krubitzer

POSITION TITLE
Professor, II

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

<table>
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<th>INSTITUTION AND LOCATION</th>
<th>DEGREE (if applicable)</th>
<th>YEAR(s)</th>
<th>FIELD OF STUDY</th>
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<td>Pennsylvania State University, PA</td>
<td>BS</td>
<td>1983</td>
<td>Comm. Disorders</td>
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<td>Vanderbilt University, Nashville, TN</td>
<td>PhD</td>
<td>1989</td>
<td>Physiol. Psych.</td>
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<td>University of Queensland, Australia</td>
<td>Post-doc</td>
<td>1992</td>
<td>Cortical Organization</td>
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A. Positions and Honors

Professional Experience

1993–1995 ARC Research Fellow, Vision, Touch and Hearing Research Centre, Department of Physiology and Pharmacology, University of Queensland.
1995-1999 Assistant Professor, Department of Psychology, and Center for Neuroscience, University of California, Davis.
1999-2001 Associate Professor, Department of Psychology, and Center for Neuroscience, University of California, Davis.
2001-2003 Full Professor I, Department of Psychology, and Center for Neuroscience, University of California, Davis.
2003 Full Professor II, Department of Psychology and Center for Neuroscience, University of California, Davis.

Honors and Awards

1987 Kreig Cortical Scholar Award, Cajal Club
1996 C. L. Herrick Award, American Association of Anatomists
1998 MacArthur Fellowship, MacArthur Foundation
2002 The James McKeen Cattell sabbatical fellowship
2003 Bloedel Visiting Scientist Fellowship, University of Washington

B. Selected Peer-reviewed publications or manuscripts in chronological order (from PHS 398/2590 (Rev. 05/01) Page 7 Biographical Sketch Page 8)


C. Research Support

Ongoing Projects
The somatosensory cortex and thalamus

Principal Investigator: Leah Krubitzer

Agency: N.I.N.D.S (NIH). RO1 NS35103-01A1
Period: 2001-2004
The objective of this project is to examine the cortical and thalamic organization and connections of somatosensory areas in the lateral sulcus and posterior parietal cortex of Old World and New World monkeys using electrophysiological, neuroanatomical, and histochemical techniques. There is no overlap with this application.

The role of the somatosensory cortex in affective social relationships

Co-Principal Investigator: Leah Krubitzer
Agency: NIH. R21 MH066756
Period: 2002-2004
The objective of this project is to examine the cortical somatosensory areas involved in affective social behavior, particularly those that require body contact. These studies are performed in New World titi monkeys and neuroanatomical, electrophysiological, behavioral and lesion techniques are used to determine the organization, connectivity and function of anterior parietal and posterior parietal areas that contribute to social behaviors that require a large sensory component. There is no overlap with this application.

How does evolution build a complex brain

Principal Investigator: Leah Krubitzer (PI)
Agency: [Private Source]
The goal of these experiments is to determine how particular developmental cascades are altered over time to produce variable phenotypes. These experiments include comparative studies of gene expression in highly derived mammals (echolocating bats and naked mole rats), cortical manipulation studies in Monodelphis domestica. There is no overlap with this application.

Linking Functional Imaging, Neurophysiology & Anatomy

Consultant: Leah Krubitzer
Agency: NINDS
Period: 2003-2006
The objective of these experiments is to validate a number of non-invasive imaging techniques including FMRI, MEG and DTI. This will be done in macaque monkeys by combining these techniques with electrophysiological recordings and neuroanatomical techniques in the same animals. There is no overlap with this application.

Completed Projects
The somatosensory cortex and thalamus

Principal Investigator: Leah Krubitzer
Agency: N.I.N.D.S (NIH). RO1 NS35103-01A1
Period: 1997-2000
The role of the somatosensory system in intra-manual and bilateral coordination of the hands

Principal Investigator: Leah Krubitzer
Agency: Private Source
Period: 1997-2000
The objective of this project was to examine the cortical organization and connections of somatosensory areas, such as S2 and PV, which are involved in manual dexterity and sensorimotor integration.

A combined fMRI study in monkeys and humans

Principal Investigator: Leah Krubitzer
Agency: Private Source
Period: 1999-2001
The objective of this project was to use fMRI techniques in humans, and combined fMRI and electrophysiological techniques in monkeys to establish viable monkey models for understanding the details of connectivity and functional organization of cortical areas in the human somatosensory cortex.

Comparative gene expression in highly derived mammals: Genetic and activity dependent mechanisms involved in cortical field development

Principal Investigator: Leah Krubitzer
Agency: Faculty Research Grant Program, University of California, Davis
Period: 2002-2003
The goal of this project was to examine the neocortex in several highly derived species that have unique neocortical organizations, such as echolocating bats and naked mole rats, and compare this organization with that of the mouse. In embryonic and neonatal animals we will examine patterns of gene expression in these mammals and compare them with that of the mouse. Because the cortical derivations appear to be largely guided by differences in peripheral sensory receptor arrays and use, these comparisons will allow us to unravel the specific contribution of genes and patterned activity in cortical field specification.

Evolution of the Neocortex: Comparative and developmental studies

Principal Investigator: Leah Krubitzer
Agency: Private Source
Period: 2002-2003
The goal of this project was to examine the mechanisms that contribute to cortical field specification in development and evolution. Cortical and peripheral manipulations are made in developing nervous systems to examine the effects of such changes on the function and connectivity of cortical fields.
BIOGRAFICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.

Follow this format for each person. DO NOT EXCEED FOUR PAGES.
BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow the sample format for each person. DO NOT EXCEED FOUR PAGES

Personal Info
RESOURCES

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Under "Other," identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

PI Resources (Dr. Leah Krubitzer)

Laboratory: The laboratory of the PI consists of 1600 square feet in a building that is part of the University of California, Davis, Center for Neuroscience (CNS). The space includes a large histology laboratory, a surgery and an electrophysiological recording room designed for multicellular recording experiments, a microscope room that is used for image analysis and data reconstruction, two perfusion stations, a workbench devoted to in situ hybridization experiments, and a data analysis room with desks and computers. Shared facilities include a photography room and equipment, laser printers, and a color pictography printer (Fujix 3000) for making journal ready prints of line drawings and continuous tone images of biological material.

Clinical: NA

Animal: The macaque monkeys will be housed at the CNPRC at UC, Davis, which is fully staffed with experienced animal care technicians and veterinarians.

Computer: In the laboratory of the PI, 16 workstations are currently in use for word processing, data analysis, microscopic image analysis, graphic reconstruction, and digital imaging of brains during surgery. These include 4 Macintosh G4’s, 3 Macintosh G3’s, 1 Power Mac, 6 Pentium PCs, 1 Celeron PC, and 1 Silicon Graphics O2 workstation with color graphics monitor for fMRI data analysis and display. All computers have 17” – 20” monitors. We also have seven ZIP drives, 1JAZZ drive, 3 CD-RW’s and 2 DVD + RW + R with + CD-RW drives for data storage, and a Microtek scanner (Scan Maker X6) to load images for digital image processing. All the necessary computer software for running experiments, data analysis and manuscript preparation are available in the laboratory (e.g. NIH image, Adobe Photoshop, Canvas, Word, Excel, PowerPoint).

Recently we have purchased the Amira 3.1 software package (TGS, Inc; San Diego, CA) and a Dell 8250 workstation (Dell; Round Rock, TX). Together they serve as a state of the art image-processing tool that is specialized for very large, three-dimensional data sets. Amira can be used to import hundreds of digital images of serially sectioned neural tissue stained for various neuroanatomical markers, anatomical tracers, in situ hybridization, or reconstructed tissue containing X/Y stage encoded cell position information, architectonic boundaries and/or gene expression patterns. Amira automatically aligns these images to generate a three-dimensional neural structure, and then labels various selected features within the reconstruction. The three-dimensional reconstruction may then be digitally "sliced" in standard or arbitrary planes of section, in order to best illustrate the features chosen by the user. This type of manipulation allows the user to visualize and measure nearly any perspective of the anatomical organization of the neural structures under investigation.

Currently all of the computers in the Center for Neuroscience are networked. The Center employs one computer technician who maintains CNS networks as well as all associated hardware.

Office: The PI has a private office next to the laboratory at the CNS, as well an additional office to house postdocs. Office space for graduate students is available in the laboratory of the PI at the CNS, as well as in the Department of Psychology.

Secretarial and administrative support is provided by the Center for Neuroscience and the Department of the Psychology. Office supplies are supplied by the Center, as is domestic mail and photocopy services.
**Major Equipment:** The PI has an electrophysiological recording laboratory that includes a preamplifier, amplifier, graphic equalizer, speakers, oscilloscope, stereotaxic frame, micromanipulator, a stepping microdrive, an hydraulic surgery table, surgical lights, a Zeiss OPMI 6 CFC surgical microscope, an anesthetics machine, a vaporizer and large and small animal ventilators. The laboratory is equipped with somatosensory and visual stimulation equipment necessary for mapping methods to be used in the proposed experiments. Also available are digital cameras coupled to computers for taking real time or still images of the brain.

The histology laboratory has two fume hoods, three microtomes, refrigerators, freezers, an in situ hybridization oven, dishwasher, scales, shakers, stirrers, glassware, and all other equipment necessary for performing the tissue processing associated with the proposed experiments. Finally, the laboratory houses a Zeiss continuous zoom SV6, a Zeiss axioskope with lightfield, darkfield, and fluorescent capabilities, and three Nikon Eclipse E400 fluorescent microscopes. All are compatible with a Spot RT slider digital camera (color and monochrome), an Optronics video camera, and a Pixera Professional: 1.2 million pixel digital camera system, display, keyboard and PC for data imaging and analysis. The Zeiss axioskope and Nikon microscopes are all equipped with a digitizer, and an X/Y stage encoding system, coupled to a PC, for reconstruction of labeled cells and a HP color plotter/printer. For chronic experiments and acute experiments, the surgical/electrophysiological laboratory of the PI has been structurally modified to meet EH&S and Government standards for work on animals.

**Other:** The California National Primate Research Center (CNPRC) at UC, Davis is a wonderful resource, and is one of the few primate centers in the country. The CNPRC currently houses over 2,500 primates and has a staff of trained veterinarians and animal technicians to care for the animals. In addition, there are a number of faculty members currently doing a wide range of animal behavior studies. These faculty are available for consultations and helping to troubleshoot problems.
A. Specific aims.

The objective of this research is to uncover the cortical contribution of area 5 to directed reaching, grasping, manual dexterity, bilateral coordination of the hands, and interhemispheric transfer of information. To achieve this goal, we will determine the full complement of cortical, interhemispheric, and thalamic connections of area 5 and adjacent somatosensory area 2 in macaque monkeys (Specific Aim 1). Then, we will assess the behavioral consequences of precisely targeted lesions to the hand and forelimb representations of posterior parietal area 5 (Fig. 1), an area proposed to be involved in directed reaching, grasping and manipulation tasks (Specific Aim 2). Finally, we will ascertain the physiological and anatomical changes that occur in the cortical organization of area 2 due to lesions in area 5. Such changes may account for behavioral recovery (Specific Aim 3).

These experiments are designed to determine if cortical area 5 is necessary for directed reaching and grasping, bimanual coordination, and interhemispheric transfer of tactile information. We hypothesize that monkeys with area 5 lesions will initially show deficits in all three intramanual and bimanual tasks (see below), and that there will be significant recovery of at least some of these tasks. We predict that this recovery of function will be due, in part, to substantial reorganization of area 2, which is heavily interconnected with area 5. Thus, anatomical studies will be critical for interpreting the behavioral results. These experiments will serve as the foundation for future studies investigating how area 5 neurons could encode the necessary stimulus attributes for complex and naturally occurring behaviors.

While we appreciate that neurons in area 5 respond optimally in an awake, behaving animal (e.g. Mountcastle et al., 1975; Iwamura and Tanaka, 1996), the goal of these experiments is not to characterize the response properties of neurons in area 5. Rather, we will utilize multilet mapping techniques in anesthetized animals to achieve our specific aims. These techniques will allow us:

1. To accurately place injections in areas 5 and 2 in normal animals, and in area 2 in post-lesioned animals (Specific Aim 1).
2. To make lesions that are restricted to area 5. (Specific Aim 2).
3. To determine the detailed organization of area 2 in a number of normal animals, so that this organization can be compared with the cortical organization of area 2 after lesions of area 5, and after behavioral recovery (Specific Aim 3).

**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AIP</td>
<td>anterior intraparietal area</td>
</tr>
<tr>
<td>CL</td>
<td>central lateral nucleus</td>
</tr>
<tr>
<td>CM</td>
<td>centre median nucleus</td>
</tr>
<tr>
<td>LP</td>
<td>lateral posterior nucleus</td>
</tr>
<tr>
<td>MD</td>
<td>medial dorsal nucleus</td>
</tr>
<tr>
<td>MGd</td>
<td>medial geniculate nucleus (dorsal division)</td>
</tr>
<tr>
<td>M1</td>
<td>primary motor cortex</td>
</tr>
<tr>
<td>Pa</td>
<td>anterior pulvinar</td>
</tr>
<tr>
<td>PM</td>
<td>premotor cortex</td>
</tr>
<tr>
<td>PRR</td>
<td>parietal reach region</td>
</tr>
<tr>
<td>PV</td>
<td>parietal ventral area</td>
</tr>
<tr>
<td>PR</td>
<td>rostrolateral parietal area</td>
</tr>
<tr>
<td>SMA</td>
<td>supplementary motor area</td>
</tr>
<tr>
<td>S2</td>
<td>second somatosensory area</td>
</tr>
<tr>
<td>VA</td>
<td>ventral anterior nucleus</td>
</tr>
<tr>
<td>VL</td>
<td>ventral lateral nucleus</td>
</tr>
<tr>
<td>VIP</td>
<td>ventral posterior nucleus (inferior)</td>
</tr>
<tr>
<td>VPs</td>
<td>ventral posterior nucleus (superior)</td>
</tr>
<tr>
<td>VS</td>
<td>ventral somatosensory area</td>
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**Figure 1.** The organization of somatosensory cortex in macaque monkeys. Area 5 (dark green) is the focus of the proposed studies, and is located on the rostral bank of the intraparietal sulcus (IPS) and wraps onto the crown of the posterior post central gyrus. Adjacent area 2 (light green) will also be examined. Areas of the lateral sulcus (orange) were explored in the previous renewal. Sulci are opened and shaded in grey.

**Specific Aim 1:** Determine the cortical, interhemispheric and thalamic connections of areas 5 and 2 in macaque monkeys. Neuroanatomical tracers will be injected into electrophysiologicaly defined locations in areas 5 and 2, and the complete set of connections, including connections with some of the electrophysiologicaly identified target regions in the ipsilateral and contralateral hemisphere, will be examined for each field. These neuroanatomical tracing experiments will be of two types:

a) Different portions of the hand and forelimb representation will be injected with different neuroanatomical tracers in area 5 or area 2 in the same animal so that similarities and differences in effenent and afferent connections across body parts within a field can be determined (see Fig. 17).

b) The same body part representation will be injected with different tracers in areas 5 and 2 in the same animals so that connections of similar body part representations in each field can be directly compared (see Fig. 16).

**Hypothesis 1:** Convergent thalamic and cortical inputs to area 5 as well as divergent ipsilateral and callosal outputs of area 5 are the anatomical substrate for sophisticated hand use in primates (see hypotheses 3-5). We predict that restricted locations in area 5 will receive convergent inputs from topographically diverse regions of somatosensory areas 2, 3a, S2 and PV (e.g. Fig. 17), and have convergent inputs from and divergent outputs to extrastriate visuomotor areas such as AIP and PRR, and motor areas such as...
Hypothesis 2: While area 2 is part of the processing network involved in sophisticated hand use, its role is more restricted to tactile processing than that of area 5. We predict that connections of area 2 are mainly with somatosensory cortical areas such as 3a, 3b, 1, S2 and PV of the ipsilateral and contralateral hemisphere, and with thalamic nuclei associated with proprioception such as VPs and VPI.

Results from Specific Aim 1 are critical for interpreting results from our lesion/behavioral studies (Specific Aim 2) as well as our studies of cortical plasticity (Specific Aim 3).

Specific Aim 2: Determine the behavioral consequences of precisely targeted, unilateral lesions to electrophysiologically specified hand and forelimb representations in area 5 on directed reaching, grasping and manipulation tasks, and the time course of behavioral recovery. To do this, we have developed tasks to assess:

a) The animal’s ability to reach and grasp objects placed at different horizontal locations under visually guided and non-visual guided conditions.

b) The animal’s ability to manipulate objects with both hands under visually guided and non-visual guided conditions.

c) The animal’s ability to transfer shape information processed from inputs of a single hand, to the opposite hemisphere under visually guided and non-visual guided conditions.

Hypothesis 3: Area 5 is necessary for visually guided and non-visual guided reaching. Our studies of connections of area 5 in titi monkeys and preliminary connection results in macaque monkeys support previous single unit studies in awake monkeys as well as lesion studies (see Background and Significance), and provide the anatomical substrate for a number of proposed functions of area 5. For instance, the suggestion that area 5 is involved in the kinematics of reaching and grasping is supported by the demonstration of dense connections between area 5 and premotor and motor cortex. The contention that area 5 generates a shoulder-centered coordinate system for visually guided reaching into extrapersonal space is supported by the extreme magnification of the representation of the hand, forelimb and shoulder, and the interconnections between area 5 and extrastriate visual areas such as AIP. The suggestion that area 5 is involved in intermanual transfer of information across hemispheres necessary for bilateral coordination is supported by the presence of dense connections between the hand representations of area 5 in each hemisphere, as well as callosal connections between area 5 and AIP and premotor cortex. Finally, the proposition that area 5 is involved in “intended or motivated” reaching and grasping is supported by connections between area 5 and cingulate cortex. Thus, we expect that lesions to area 5 will have devastating consequences on the animal’s ability to reach and grasp that will persist in non-visual guided reaching conditions. Specifically, we predict that immediately following the lesion to area 5, the animal will be able to reach with the ipsilesional hand under both visual and non-visual guidance, but will not be able to reach under non-visual or visually guided reaching conditions with the hand contralateral to the lesion (contralesional hand). However, the animal will regain the ability to perform this task with the contralesional hand under visual guidance, although the time required to complete the task may increase (task a).

Hypothesis 4: Area 5 is necessary for bimanual coordination of the hands. Following the lesion to area 5, we predict that the animal will not be able to manipulate objects with both hands under non-visual guided or visually guided conditions. However, the animal will regain the ability to perform this task under visual guidance, but with an increase in time to complete the task. The animal will not regain the ability to complete this task without visual guidance (task b).

Hypothesis 5: Area 5 is necessary for interhemispheric transfer of shape information across hemispheres. Following the lesion to area 5, we predict that the animal will not be able to transfer shape information across hemispheres under non-visual guided or visually guided conditions with either hand. However, the animal will regain the ability to perform this task under visual guidance with both hands, but with an increase in time to complete the task (task c).

Specific Aim 3: Determine the degree to which topographic reorganization has occurred in cortical area 2, and the changes in motor connectivity that may have occurred in area 2 due to lesions in area 5 (Figs. 2 and 3). We appreciate that the behavioral recovery that we expect to see may actually be due to plasticity at other cortical and/or subcortical sites such as areas of the lateral sulcus or the anterior pulvinar (see expected outcomes, page 43). However, area 2 was selected as a potential target area of reorganization because it receives proprioceptive inputs, has dense connections with area 5, and has callosal connections of the hand representation. These features indicate that area 2 is part of the cortical network associated with proprioception and visually guided reaching and grasping. Almost all studies that have examined cortical reorganization following removal of an area have lesioned primary sensory or motor areas and examined the consequences on higher order cortical organization. However, there is no reason to believe that feedback connectivity would have a smaller effect on cortical organization of any given field than feed.
forward connectivity. Indeed, our experiments will be the first to examine feedback plasticity in any animal. While it would be ideal to examine the effects of area 5 lesions on the organization of AIP as well as area 2, this is not feasible since we cannot consistently identify AIP using our recording techniques. On the other hand, neurons in area 2 have been demonstrated to respond robustly under recording conditions similar to those we describe in this proposal (Pons et al., 1985).

Hypothesis 6: Cortical area 2 will undergo dramatic physiological and anatomical reorganization following lesions to area 5. This plasticity could serve as the basis for the behavioral recovery we expect to see.

Physiological reorganization. We predict that lesions to area 5 will result in an expansion of portions of area 2 devoted to the representation of the hand (Fig. 2). This prediction is based on previous studies which demonstrate that lesions to motor cortex result in an expansion of representations, similar to those lesioned, of neighboring cortical fields (see Background and Significance). This physiological changes we expect to see in area 2 may be the result of rapid unmasking following the lesion (Fig. 2), and/or changes in cortical connections over time (Fig. 3). Multunit mapping studies of area 2 will be done in normal animals and post lesion animals after behavior recovery asymptotes.

Anatomical reorganization. We predict that lesions to area 5 will result in alterations in connections of area 2 over the course of behavioral recovery, and that in part, area 2 will take over some of the functions normally performed by area 5. Three types of connection changes are possible:

a) New connections may form between area 2 and other cortical areas such as PRR (Fig. 3).

b) Changes in the density of existing connections between area 2 and other cortical fields such as AIP may occur (Fig. 3). For example, preliminary studies indicate that area 5 and AIP are strongly interconnected while area 2 and AIP are weakly interconnected. It is possible that an existing weak connection may be strengthened and induce an elaboration in arborization. (Fig. 3).

c) Changes in the divergence and/or convergence of existing connections of a particular cortical field such as area 1.

Figure 2. A model of the types of anatomical connections that may account for the functional plasticity that we hypothesize to occur in area 2. The release of inhibition (via connections from area 5) of excitatory inputs from areas 1 and the anterior pulvinar could result in an expansion of the hand representation in area 2.

Figure 3. A model of the types of new connections that may form following ablations of area 5. For example, new connections may form between area 2 and the parietal reach region (PRR). Changes in the density of existing connections between area 2 and AIP, or changes in the divergence or convergence of connections between area 1 and 2 may also occur.

In these studies, injections of different neuroanatomical markers will be placed into different electrophysiologically identified body part representations in area 2 in both normal (Specific Aim 1) and post lesion animals, after behavioral recovery asymptotes. While we will chart the time course of behavioral recovery, it is beyond the scope of this study to chart the time course of physiological and anatomical plasticity in area 2.
The ability to use our hands to manipulate the world around us distinguishes human and non-human primates from most other mammals. Indeed, the refinement of the hand and the emergence of cortical areas associated with hand use is one of the hallmarks of primate evolution (Napier, 1960; 1962; Culham and Kanwisher, 2001). Despite the importance of these abilities, and the extreme to which they have been developed in humans, very little is known about the neural control of manual behaviors. Further, there is only a rudimentary understanding of the cortical circuitry underlying hand use, with very little understanding of the specific behavioral deficits which arise when specific areas involved in these abilities are ablated. Finally, only a few studies have examined the physiological and anatomical plasticity that occurs in adjacent cortical areas following loss of a cortical regions (e.g. Nudo and Milkin, 1996; Frost et al., 2003; Dancause et al., 2003). The goal of the studies proposed is to fill this gap by providing a more complete understanding of one of the areas involved in manual abilities, posterior parietal area 5.

**Posterior parietal area 5 and anterior parietal area 2 in primates**

**Area 5.** Posterior parietal cortex has recently been subdivided into a number of cortical fields including areas 5, AIP, LIP, MIP, VIP and V6 (PRR, see Anderson et al., 1997, and Culham and Kanwisher, 2001), and these fields are thought to form a processing network involved in generating eye and/or body centered coordinates for reaching, monitoring limb location during visually guided and non-visual guided reaching tasks, converting sensory locations into motor coordinates for intentional movement, and perceiving the movements of the body in extra personal space (e.g. Mountcastle et al., 1975; Mountcastle, 1975; Iwamura and Tanaka, 1996; Kalaska et al., 1997; Snyder et al., 1997; Andersen et al., 1997; Graziano et al., 2000; Gregoriou and Savaki, 2001).

The experiments of this proposal will test the hypothesis that one of these posterior parietal areas, area 5, is critical for visually guided and non-visual guided reaching, bilateral coordination, and interhemispheric transfer of information by determining the effects of small lesions restricted to the hand/forelimb representation of area 5 on these behaviors.

Several consistent features of area 5 have emerged regarding its location, organization, receptive field characteristics, and the submodality of receptors to which neurons are responsive. Although traditional architectonic studies propose that area 5 is a very large cortical area that occupies the entire rostral bank of the IPS and much of the caudal post-central gyrus (e.g. Brodmann, 1909), modern electrophysiological and anatomical studies indicate that area 5 is much smaller and resides in the middle of the rostral bank of the IPS and folds around the sulcal crown to spread onto the adjacent postcentral gyrus (Fig. 1; e.g. Pons et al., 1985; Iwamura, 2000 for review). Area 5 is dominated by the representation of the hand and forelimb; neurons in area 5 have contralateral, ipsilateral, and bilateral receptive fields (particularly on the hand and forelimb), and most neurons respond to stimulation of deep receptors of the skin and joints (Mountcastle et al., 1975; Taoka et al. 2000; Iwamura et al., 1993; 1994; 2002; see Iwamura, 2000 for review). Single unit studies in macaque monkeys indicate that area 5 is involved in coordinating or programming intention of movement (Snyder et al., 1997; Debowy et al., 2001), and that area 5 generates body or shoulder centered coordinates for reaching, rather than eye centered coordinates (Perraina and Bianchi, 1994; Laquintin et al., 1995; see Wise et al., 1997 for review). Further, area 5 appears to be involved in the kinematics (e.g. spatiotemporal coordinates) rather than the kinetics (e.g. load and force of muscle) of reaching (Kalaska, 1996; Wise et al., 1997). Our recent studies in New World titi monkeys, as well as preliminary findings in macaque monkeys, indicate that many neurons in area 5 can be driven by visual stimulation (Fig. 7). Colby and Duhamel (1991) identified visually responsive neurons on the rostral (medial) bank of the IPS. Although they term this region MIP, it actually looks as though the location of their recording (based on a schematic drawing) is what is currently considered as area 5. It is difficult to localize the site of their recordings since no figures are provided to specify their electrode location. Taken together, all studies indicate that neurons in area 5 have a number of functional properties that are important for reaching, grasping, and manual and bimanual coordination.

An understanding of the connections area 5 is critical for appreciating how area 5 generates an internal representation of self, and uses this representation as a coordinate system for sophisticated hand use. However, virtually nothing is known about the connections of area 5 in macaque monkeys. Only one study used electrophysiological guidance to place injections in area 5, and in this study, only one injection in one animal was performed, and the injection spread into area 2 (Pons and Kaas, 1986). Further, only local connections were examined. Studies of vestibular processing in human and non-human primates indicate that cortex at the juncture of areas 5 and 7b projects to vestibular brainstem nuclei, contains neurons which respond to optokinetic and vestibular stimulation, and is interconnected with other areas of the neocortex that process vestibular information (Akbarian et al., 1988; 1993; 1994; Guldin et al., 1992; Brandt and Dieterich, 1999; Loebl et al., 1999; see Guldin and Grissler, 1998 for review). To our knowledge, there are no other studies that have made injections restricted to electrophysiologically identified area 5 and examined the full compliment of cortical or subcortical connections. Rather, previous studies examined the connections of large portions of cortex, that included area 5, and the degeneration techniques utilized were not as sensitive as modern neuroanatomical tracing techniques (e.g. Jones and Powell, 1970; Pandya and Seltzer, 1982).

We were surprised that our studies in titi monkeys and preliminary studies in macaque monkeys represent the most complete and specific data regarding connections of this field. Although limited the data indicate that area 5 is densely connected with areas 1,
Area 2 There are several studies that examined the topographic organization and response properties of neurons in area 2 (e.g. Hyvärinen and Poranen, 1978; Pons et al., 1985; Taoka et al., 1998; 2000; Iwamura et al., 2002). These studies indicate that neurons in area 2 respond well to stimulation of deep receptors, although in some portions of area 2 neurons also responded to cutaneous stimulation (e.g. Pons et al., 1985; Ageranoti-Bélanger and Chapman, 1992) and that receptive fields are relatively large (sometimes bilateral) when compared to areas 3b and 1 (e.g. Taoka et al., 2000; Iwamura et al., 2002). Further, in awake behaving monkeys, neurons in area 2 are active during self-initiated reaching and grasping. Pons and colleagues (1985) have demonstrated that neurons in area 2 can be reliably driven in anesthetized animals, and that area 2 contains a complete representation of deep receptors on the contralateral body, although the topographic organization is not as precise as in areas 3b and 1. Studies of connections of area 2 in which injections were placed under electrophysiological guidance indicate that this field is connected with other somatosensory cortical areas such as 3b, 1, 3a, and S2, as well as with M1 and area 5 (Pons and Kaas, 1986). Area 2 receives thalamic input from divisions of the ventral posterior complex (VP, Vpi, and Vps) as well as the anterior pulvinar (Pa).

Our proposed studies of connections of both areas 5 and 2 are important for several reasons. First, the full complement of connections of area 5 have never been described for the macaque monkey. An appreciation of the connections of any cortical field provides insights into the function of that field (see hypothesis 2), and its relationship to other fields in a particular processing network. For area 5 in particular, understanding its full complement of connections will allow us to interpret our behavioral results, and better explain the loss of particular manual abilities. While some of the connections of area 2 have been described in macaque monkeys, these previous studies cannot serve as adequate controls for our studies of area 2 connections in animals which received lesions to area 5. Our goal is to compare connections of area 2 in normal animals with animals that received lesions to area 5 and have undergone behavioral recovery. Thus, in both normal and lesioned animals it is necessary to use the same tracer, in the same body part representation, and to quantify the injection site, the location of transported tracer, and cell and terminal bouton density in an effort to understand the anatomical plasticity that may occur in the neocortex after insult of neighboring cortical regions. These studies represent one of only a few that investigate the neurophysiological and neuroanatomical correlates of behavioral recovery after cortical insult. Also, they are the first to examine the effects of higher order cortical area lesions (area 5), on lower order areas, such as area 2. While this is not the goal of the present investigation, examining the effects of feedback on the organization of lower order cortical fields, and the possibility that feedback plasticity does occur after lesions to higher order fields is exciting in its own right, and certainly would provide important insights on normal brain organization and function.

Behavioral deficits resulting from cortical lesions of parietal cortex

One way in which the function of cortical fields can be explored is to lesion a particular region or area, and examine the resulting behavioral consequences. This method was a prominent means for exploring cortical function in the latter half of the 20th century. Unfortunately, this technique is not widely used today, possibly due to lack of specificity in lesion placement in these early experiments, and the associated difficulty of interpreting the results. Despite this, lesion techniques still offer the advantage of directly determining the cortical basis for discrete behaviors, something that can only be inferred from single unit recording studies. Indeed, the contribution of these types of studies to our understanding of cortical function, as well as cortical processing strategies, is well exemplified in the work of Mishkin and colleagues (1979; 1982; 1983). Their studies of visual cortex lesions in monkeys revolutionized our understanding of how cortex processes different types of inputs, and established the framework of modern theories of visual processing.

With regard to somatosensory cortex, a number of lesion studies have uncovered some of the functions that certain regions or cortical areas assume, and have laid the groundwork for more recent single unit recording studies in posterior parietal cortex. For example, lesions to the anterior parietal strip (3b, 1, 2 and possibly portions of area 5) result in deficits in flutter frequency discrimination (Lamotte and Mountcastle, 1979), while lesions to posterior parietal cortex, including areas 2, all of the IPS, 7a and 7b, result in misorientation of fingers, and deficits in reaching under visually and non-Visually guided conditions (Lamotte and Acuna, 1978). Studies in which lesions were more restricted demonstrate that areas 3a and 3b result in severe deficits in a variety of tactile discrimination tasks (Randolph and Semmes, 1974), lesions of area 1 affect texture discrimination (Randolph and Semmes, 1974; Carlson, 1981), and lesions of area 2 affect the discrimination of size and curvature of objects (Carlson, 1981). Although limited, studies in humans with lesions to the anterior portion of the postcentral gyrus (in areas 3b, 1 and 2) support studies in monkeys in that they demonstrate that individuals suffer from impairments in discrimination of object size and shape (Roland, 1976).
There are a few restricted lesion studies in posterior parietal cortex in monkeys that incorporated area 5. For example, studies in which unilateral lesions included areas 5, MIP and 7b demonstrated that animals had deficits in coordinating arm velocity with hand velocity, that the postural relationship between the arm and wrist was disrupted, and that there were disruptions in coordinating the hand in shoulder centered space (Rushworth et al., 1997). However, these lesions did not affect the range or velocity of movements, or the hand’s trajectory. In a related investigation in which areas 5, 7b and MIP (which may have also included V6 or the parietal reach region, PRR) were bilaterally ablated, the monkey had deficits in reaching to the same target under different starting positions (Rushworth et al., 1998). Finally, a study in green monkeys in which bilateral ablations were made to area V6A (but may have incorporated portions of area 5) demonstrates that monkeys show a reluctance to move, and have deficits in reaching, grasping, and wrist orientation (Battaglini et al., 2002). While most other studies which examine the effects of posterior parietal lesions on behavior are difficult to interpret because the size of the lesion was so large, they do indicate that lesions to posterior parietal cortex spare tactile discrimination abilities (Murray and Mishkin, 1984; Brown et al., 1983).

There are a number of studies in humans which examine the deficits that occur with insults to posterior parietal cortex. These studies indicate that the most severe deficit, termed spatial hemineglect, is in coding spatial location of objects within a particular frame of reference (see Robertson and Ralf, 2000; Behrmann, 2000 for review). While the data indicate that there are several egocentric frames of reference, including that of the limb of the body, there is little data on areas of posterior parietal cortex that contribute to this frame of reference. The major problem with lesion studies in humans is the same as those associated with lesion studies in monkeys; the lesions are extremely large and encompass a number of cortical areas. Therefore, it is difficult to interpret which area is associated with which aspect of the deficit. Also, most of these human lesion studies examine the consequences on visual processing and spatial attention (see Behrmann, 2000 for review).

The lesion studies in monkeys are interesting in that they support single unit studies in posterior parietal cortex, and in some instances extend them. However, these studies were confounded because the lesions were extremely large, the cortical field ablated was not physiologically determined, and the lesion incorporated a number of cortical fields. We have demonstrated in titi monkeys that we can make very small lesions in area 5 (~1 mm x 2 mm) and that such a lesion can have an enormous impact on the animal’s ability to reach for and grasp intended objects. In the proposed experiments we will extend our lesion studies to macaque monkeys, and use refined behavioral tasks to tease apart different aspects of reaching, grasping, bimanual coordination, and intermanual transfer abilities.

**Intermanual transfer of information across cerebral hemispheres**

Throughout the 1960s, 70s and 80s, a few laboratories examined the effects of sections to the corpus callosum on intermanual transfer of information. Although these studies did not attempt to examine the role of any particular cortical field on intermanual transfer, the questions addressed were extremely important since mammals, and primates in particular, use both hands in a coordinated fashion to manipulate and explore objects or accomplish a task. These studies document several important observations. The first is that the deficits in intramanual transfer abilities resulting from sections to the corpus callosum and associated commissures were directly related to the size and placement of the lesion (Hunter et al., 1976; Myers and Ebner, 1976). If lesions were made to the posterior half of the corpus callosum, animals showed no transfer of information on shape or roughness discrimination tasks. This finding is important because areas of posterior parietal cortex send axons through the posterior portion of the corpus callosum (Seltzer and Pandya, 1983). The second finding was that transfer deficits resulting from sections to the corpus callosum depended on the type and difficulty of the task performed (Manzoni et al., 1973). For example, tasks which required the animal to discriminate and transfer information about two distinct objects were performed well, while tasks which required the animal to transfer subtle information about the size and shape of an object, were performed poorly. Finally, access to visual information when performing the task had a large effect on information transfer across hemispheres (Kohn and Meyers, 1969). If animals were allowed to view the manual task to be transferred they performed close to normal behavior. However, they could not transfer information without visual guidance, as normal animals could. Interestingly, lesions of the corpus callosum did not change hand preference in monkeys (e.g. Lehman, 1972).

Studies of tactile and tact uomotor transfer in humans, which have undergone complete section of the corpus callosum, or partial sections of the posterior portion of the corpus callosum indicate that the effects of these lesions were dependent on the portion of the callosum that was removed, the difficulty of the task, and the presence of visual monitoring. For example, complete section of the corpus callosum (Geffen et al., 1985) or sections to the portion of the callosum through which axons connecting the posterior parietal cortex of each hemisphere travel (Geffen et al., 1985; Risse et al., 1989) result in an inability to perform cross-localization intermanual tasks, but intramanual localization remains intact (Geffen et al., 1985; Lassonde et al., 1986; Risse et al., 1989). Other studies demonstrate that with sections of the posterior corpus callosum, individuals cannot perform posture matching tasks (kinesthesis) in which they are required to match the position of one forelimb and hand with the opposite forelimb and hand in the absence of visual guidance (Risse et al., 1989). Finally, late adolescents with sections of the corpus callosum performed poorly on transfer of information regarding object shape, and the magnitude of the effect was dependent on the difficulty of the task. For
instance, these individuals performed better on intermanual matching tasks involving familiar objects, and performed less well on matching 2-dimensional and 3-dimensional shapes (Lassonde et al., 1986).

These previous studies indicate that the posterior parietal cortex transfers crucial information between hemispheres to allow bimanual coordination of the hand. Unlike anterior somatosensory fields (3a, 3b and 1) in which the hand representation is mostly acallosal, area 5 receives interhemispheric input in the expected location of the hand representation (Shanks et al., 1985). Our studies in tita monkeys and our preliminary work in macaque monkeys indicate that the hand representation of area 5 has callosal connections to the contralateral area 5 and premotor cortex. This suggests that this is one of the first somatosensory cortical areas within the cortical hierarchy to be involved in coordinating behaviors between the hands, and in the interhemispheric transfer of information necessary for limb and hand coordination. The available data in humans is consistent with work in monkeys in that visually guided reaching and grasping tasks activate cortex that incorporates areas in and around the IPS (Kertzman et al., 1997; Culham et al., 2003, see Culham and Kanwisher, 2001 for review). Our preliminary data in humans also indicates that cortex on the rostral bank of the IPS contains a field that is dominated by the representation of the hand and is active during visually and non-visually guided reach tasks (2001b; Hinkley et al., 2004). Further, fMRI studies in callosotomized patients demonstrate a lack of ipsilateral activation to hand stimulation in posterior parietal cortex which is present in normal individuals (Fabri et al., 1999; 2001). This indicates information processed from one hand reaches the contralateral hemisphere via posterior parietal cortex.

Thus, area 5 is ideally suited to be involved in bimanual coordination and interhemispheric sotmication of somatic information, a role that has not been considered previously. The experiments in this proposal will investigate this issue by examining the interhemispheric connections of electrophysiologically identified representations of the hand and forelimb in area 5 with electrophysiologically defined representations in area 5 and surrounding fields. Further, we will determine effects of unilateral lesions of electrophysiologically defined hand and forelimb representations of area 5 on both bimanual dexterity tasks, as well as task involving interhemispheric transfer of tactile shape information.

**Functional and connectional changes in the neocortex resulting from peripheral and central nervous system insult**

Despite the fact that a large portion of individuals that sustain cortical damage due to trauma or stroke show recovery of a number of motor, sensory and cognitive abilities, we have little understanding of the underlying mechanisms that generate this plasticity. A better understanding of the neural mechanisms that support such recovery is critical for effective rehabilitation. On the other hand, a number of studies have shown that peripheral injuries, as well as practice or specialized use of a sensorimotor system can generate substantial changes in the functional organization of cortical fields in the form of expansions and contractions of cortical maps. These map changes are believed to underlie, at least in part, the functional recovery of perceptions and behaviors following such injuries, or the improved performance following practice. While the physiological changes that follow peripheral sensory damage or use have been well documented, only a few studies have examined the anatomical changes that occur in the neocortex, which may account for the observed plasticity. For example, visual cortex reorganization following retinal lesions (e.g. Kaas et al., 1990; Calford et al., 1999, 2003) is at least partly mediated by local axonal sprouting into the denervated representation (e.g. Darian-Smith and Gilbert, 1994). Sprouting of cortical neurons has also been demonstrated in the somatosensory cortex of macaque monkeys after large peripheral denervations (Florence et al., 1998).

As noted above, there is only limited data on functional and anatomical plasticity that occurs following cortical damage. Work in the Nudo laboratory in squirrel monkeys has examined the functional and anatomical plasticity of cortex that is adjacent to lesions in motor cortex (M1). This work is similar to the work proposed in this study in several respects. First, they electrophysiologically identified the cortical area to be to be lesioned. Second, these investigators have microstimulated cortex around the lesioned area to determine the changes in cortical organization that have occurred. They find that cortex immediately adjacent to the lesion reorganizes in that similar representations contract (digits of the hand), and more distant, but related representations expand (such as the proximal limb representation (Nudo and Millikin, 1996). In cortex that is relatively close, but not adjacent (i.e. the ventral premotor cortex; vPM), they find that similar representations to that lesioned (e.g. digits) expand (Frost et al., 2003). Third, they find that with lesions in M1, vPM forms new connections with anterior parietal cortex (Dancause et al., 2002; R. J. Nudo, personal communication). Finally, recent work indicates that rehabilitative training after motor inants in the hand representation, in addition to peri-infarct stimulation, increases the hand representation in adjacent motor cortex. Such expansion appears to be responsible for the behavioral recovery observed in these animals (Plautz et al., 2003). These studies have proven very useful when considering our expected outcomes, and were the impetus for exploring the possibility that changes in cortical connectivity may occur in our animals after our lesions.

In summary, while area 5 has been traditionally viewed as a unimodal somatosensory area involved in manual dexterity and reaching tasks, there is still very rudimentary knowledge of the anatomical connections, and the basic functions observed by this area. While there are several intriguing findings suggesting a prominent role of this field in complex uni- and bimanual behaviors, this has yet to be experimentally verified. The experiments in this proposal will tackle this complex issue, first by defining the neuroanatomical connections of this area, second by assessing the consequences of restricted lesions of area 5 on a variety of
C. Progress Report and Preliminary Studies.

Progress report
We have made excellent progress since our funding began in September of 2000. We have completed a number of projects, have begun to effectively utilize new techniques for analyzing and quantifying data, and have generated preliminary data on studies we propose in this application. We have eight publications and one review directly related to this renewal, one review on the evolution of human cortex related to this renewal (from a total of 16 publications since September of 2000), and 9 abstracts related directly to this application (from a total of 25 since September, 2000). Published work includes studies of the organization of area 3a and its cortical and thalamic connections in marmoset monkeys (Appendix 1 and 2); a study of the cortical organization of area 3a in macaque monkeys (Appendix 3); studies of cortical and subcortical connections of areas S2 and PV in macaque monkeys (Appendix 4 and 5); and studies in human and non human primates using functional magnetic resonance imaging (fMRI) and magnetoencephalography (MEG) techniques (Appendix 6-8). We have completed studies of the organization and connections of area 5 in titi monkeys [Submitted see publication 15, and preliminary results]. Finally, there have been two new techniques that we have begun to utilize in the laboratory to examine both human and nonhuman primate brains. The first is the implementation of a 3D data reconstruction system for use in histologically processed brains of non-human primates. The second is the use of diffusion tensor imaging (DTI) to examine, non-invasively, the connections of particular cortical fields in humans. Although these techniques are not yet represented as published papers, they are part of a large effort in our laboratory to develop new, state of the art tools for examining brain organization and connections in human and non human primates.

Publications since previous application (Bold denotes those directly related to this grant, * denotes publications which appeared on the cover of journals)

1. Personal Info
4. Personal Info
5. Personal Info
8. Personal Info
11. Personal Info
14. Personal Info
15. Personal Info, Submitted
Relevant abstracts (those that have been already published in full are not listed).

1. Personal Info
2. Personal Info
3. Personal Info
4. Personal Info
5. Personal Info
6. Personal Info
7. Personal Info
8. Personal Info
9. Personal Info

**Area 3a in non-human primates (publications 6, 7 and 14; appendix 1-3).** Our studies of area 3a in both marmoset and macaque monkeys are the first complete mapping studies of this field in any primate (Huffman and Krubitzer, 2001a; 2001b; Krubitzer et al., 2004). The results from both studies are the first to demonstrate that the organization of area 3a is generated as a function of the use of particular body parts (particularly the hand), more so than in area 3b. Further, both cortical and subcortical connections of area 3a indicate that it is closely associated with the motor system and regions of posterior parietal cortex, rather than the traditional view that it is primarily involved in somatosensory processing.

**Areas S2 and PV in non-human primates (publications 8 and 11; appendix 4 and 5).** In this series of studies we have examined the cortical and thalamic connections of S2 and PV in macaque monkeys. The results from these studies indicate that both fields are part of a pathway involved in object recognition, as has been previously proposed for lateral sulcus areas (Murray and Mishkin, 1984). However, unlike the visual system, ventral and dorsal pathways are not strictly segregated in that these fields also have connections with areas of posterior parietal cortex. Further, PV has connections with portions of auditory and motor cortex. Such connections could form the basis of articulation abilities by integrating somatic and acoustic inputs with the motor system.

**fMRI and MEG in human primates (publications 4 and 5, appendix 6-7).** Our goal was to determine if some of the areas in the lateral sulcus (S2 and PV) and posterior parietal cortex (area 5) are similar in both humans and macaque monkeys. Our fMRI studies in humans indicate that the organization of both S2 and PV is much like that described for macaque monkeys, and our MEG data indicate that callosal inputs from the representation of the hand are processed in these fields, but not in 3b or area 1, similar to that in macaque monkeys. A study examining the tactile and motor properties of a field just rostral to PV, the parietal rostral area (PR), is now complete and being prepared for publication (Hinkley et al., 2001; 2002).

Studies of area 5 in humans are under way and will be complete by next year. fMRI data indicate that area 5 is dominated by the representation of the forelimb and face (2000c, 2001b). Further, we find that area 5 is active during visually guided and non-visually guided reaching tasks. When activations produced from guided reaching are isolated from the purely motor component of reaching and grasping, area 5 and cortex caudomedial to it (possibly MIP) are activated (Fig. 5). This result indicates that area 5 is not specifically a visual or somatosensory area, but is a "central planner" which works in conjunction with these systems to generate a motor plan for grasping intended objects in immediate extrapersonal space, as we propose in macaque monkeys.
**fMRI and electrophysiological recordings in non-human primates (publication 1; appendix 8).** At the time this study began (1999), only a few laboratories were pursuing the relationship between fMRI and the underlying neural activity it was purported to reflect. In the same monkey, we related our fMRI results to multunit electrophysiological recording results. We found that there was only a 55% congruence between electrophysiologically determined maps and fMRI determined maps using garden-variety fMRI techniques (which is what most of the neuroscience community was utilizing at the time — and still is). We also found that the plane of greatest variance was perpendicular to the plane of major vasculature that supplied the region of interest. In short, the fMRI technique as currently utilized, to a large extent, tracks blood flow rather than specific neural activity. Needless to say, this finding was met with a good deal of opposition. However, in subsequent years, this study has been referred to as a “tour de force” and “a heroic effort” to test the validity of a widely used technique. This research was funded by our previous grant, and is currently being directed by [Personal Info] in our laboratory. Because of this work, [Personal Info] has recently received [Private Source] for testing the validity of imaging techniques.

**DTI in non-human primates (abstract 7).** One of the goals of the last application was to establish a parallel monkey–human model of cortical organization, function and connectivity of somatosensory areas of the lateral sulcus. Our published data on fMRI and MEG indicates that for some cortical fields, such as S2, PV and area 5, the macaque monkey serves as a useful model. Unfortunately, MEG only allows one to infer connectivity indirectly. A new technique, diffusion tensor imaging (DTI), has recently been established to allow one to non-invasively examine the connectivity of cortical fields. While the potential of this technique is exciting, it has never been validated. Our previous grant allowed us to pilot a series of experiments to test the validity of these techniques in macaque monkeys. In the same monkey, we have utilized fMRI, MEG and DTI techniques to examine the regions of cortex activated by cutaneous stimulation of the hand and face, the time course of activation, and the connections of this activated region respectively. We find that the connections determined using DTI are similar to those described using traditional neuroanatomical tracing techniques, at least for the very large fiber tracts. This suggests that determining the connections of particular cortical fields, such as area 5 in humans, may be possible with these non-invasive techniques. To test the validity of the connections we observed, in this same animal we will perform electrophysiological recordings and neuroanatomical connections of the “seeded area”. Preliminary results of this work have been presented at Society for Neuroscience meeting held last year in New Orleans. This work was funded by our previous grant, and is currently being pursued.

**Preliminary results**

3D data reconstruction and quantification of volume in histologically processed brains. The importance of understanding the cortical networks associated with a particular function, or the specific connections that ultimately contribute to neuronal response properties of a particular cortical field, is unquestionable. However, the inability to simultaneously examine laminar and areal patterns of connections and to quantify injection sites has placed restrictions on neuroanatomical experiments. There have been several problems associated with this issue that, until recently, have been difficult to overcome and/or too expensive to implement (e.g. NeuroLucida). The first problem is the quantification of injection sites. While it has been possible to quantify cell number and bouton density in a particular cortical field or subcortical structure for any given injection of a neuroanatomical tracer in a...
single animal, techniques for comparing cell densities across injections within and between cases have not been readily accessible. The ability to quantify our injection sites is critical for the connectional plasticity studies proposed in this application. The only way to accurately determine if new connections have formed is to compare connections of the same field in normal and post-lesioned animals. These comparisons are not valid unless the injection volume, tracer used, and representation injected is held constant. Recently we have been utilizing a new software and hardware system (Amira) that allows us to accurately combine a number of different types of data sets (e.g. connections, histochemical markers, in situ hybridization, electrophysiological recordings) onto a single, 3-dimensional reconstruction of the brain (Fig. 6). This program also allows us to make volume measurements of structures, cortical fields, representations of body parts within cortical fields, lesions, and injection sites.

Although a large amount of human interface is still necessary when using Amira, this system will revolutionize how data is analyzed in our laboratory.

Figure 6. Amira reconstruction of a macaque monkey brain (photograph top left, reconstruction top right) with injections of four different tracers into electrophysiologically identified locations (bottom). BDA (blue) has been injected into area 5, diamidino yellow (yellow) has been injected into area 2, fluororuby (red) has been injected into area 1, and fluorescein (green) has been injected into area 3b. The volume of the injections can be calculated as an absolute volume in cm$^3$, or as a percentage of the entire brain volume. For the injections above, the volumes are BDA = 0.64 cm$^3$ or 0.0042%; Dy = 0.99 cm$^3$ or 0.0055%; Pr = 0.60 cm$^3$ or 0.0040%; Fes = 0.30 cm$^3$ or 0.0020%. Thin lines in bottom figures mark cortical field boundaries determined from Nissl stained sections.

While the goal of this application is to examine connections of area 5 in macaque monkeys, these studies in titi monkeys were important for generating viable hypotheses and determining the feasibility of the lesion studies that we are proposing in this application.

Figure 7: A map of area 5 and surrounding cortex in a macaque (left) and titi monkey (right). Multiple recording sites were made on the dorsal bank of cortex rostral to the IPS, and in the rostral and caudal banks of the IPS. We find that area 5 is dominated by the hand, forelimb and shoulder representations, that neurons in this field respond to deep stimulation, cutaneous stimulation of the hand, and visual stimulation. The boundaries of fields were determined by combining electrophysiological recordings with cortical architecture (see Fig. 8). The field we term area 5 in titi monkeys was considered homologous to area 5 in macaque monkeys because of similarities in organization, connectivity, and cortical architecture.

Multunit electrophysiological recordings in both titi and macaque monkeys indicate that neurons in area 5 area respond to stimulation of deep receptors of the contralateral (and sometimes ipsilateral) body, and that area 5 is dominated by the representation of the hand and forelimb, as has been described in previous investigations (e.g. Pons et al., 1985; Takaoka et al., 1998; Fig. 7). Further, in some cases, some neurons in this field respond to visual stimulation. One previous study of area 5 noted only a very small percentage of neurons which respond to visual stimulation (e.g. Mountcastle et al., 1975), and a more recent study (Colby and Duhamel, 1991) found that neurons on the medial bank of the IPS at a rostral location respond to visual stimulation. Although they term this field MIP, we believe it is similar to what we term area 5 (see above). In both titi and
Fig. 8 Architectonically identified areas 3b, 1, 2, and 5. A) Location of areas in the central sulcus and intraparietal sulcus in horizontally sectioned, Nissl-stained tissue of macaque monkey. Boundaries of areas are marked with black arrowheads. B) Location of areas on the lateral surface and intraparietal sulcus of ttti monkey in horizontally sectioned tissue stained for cytochrome oxidase histochemistry. C) Hemisphere contralateral to that shown in panel B, also stained for CO histochemistry. This monkey received a small aspiration lesion of electrophysiologically identified area 5 six months prior to final mapping and sacrifice. White arrow and dashed line indicate the location and extent of area 5 lesion. Rostral and caudal as indicated. Laterai in all panels is at the bottom. Scale bars = five millimeters.

We also have preliminary connection data from 2 macaque monkeys (Fig. 9). Area 5 has dense connections with ipsilateral cortical areas AIP, PRR, 2, 1, PM, SMA and anterior cingulate cortex (Fig. 9), and contralateral connections with areas 5, SMA, and PM (Dishow et al., 2001; Personal Info). The strong connections observed between area 5 and motor, premotor and supplementary motor areas support a role for area 5 in the kinematics of movement (see Background and Significance). Further, in both species of monkeys the connections that exist between the hand and forelimb representations of area 5 with area 5 and PM in the opposite hemisphere support its role in coordinating inputs between the hands for bimanual dexterity.

Behavioral studies and lesions of area 5 in ttti monkeys and consequences on reaching and grasping (abstract 6). While electrophysiological and neuroanatomical studies provide some insight into how a particular field functions, lesion studies are also important tools for elucidating the function of cortical areas. We have developed methods of making restricted lesions to electrophysiologically defined portions of area 5 Personal Info. These methods circumvent the problems associated with data interpretation when lesions are very large and encompass many fields (see Background and Significance). In these studies, we trained ttti monkeys to reach for and grasp a preferred food item under visually guided and non-visually guided conditions. After the monkey reached criterion (90% correct in 25 consecutive trials), the cortex was mapped and area 5 contralateral to the trained hand was identified and lesioned (Fig. 10). After aspiration of area 5, the cortex surrounding area 5 was remapped to determine the extent of the lesion.
Unfortunately, a virus in the titi monkey colony resulted in the loss of one of our lesioned animals, and a very long illness in another animal (immediately following the lesion). However, in one of our animals we were able to examine the organization of the neocortex using electrophysiological recording techniques six months following the lesion, and after behavioral recovery. Several important observations were made that have an important bearing on the present investigation. First, the lesioned area and surrounding cortex is easily visualized, and there is little damage to the cortex other than the actual lesion (Fig. 10F). Large vasculature could be reliably identified, and smaller capillaries had grown over the lesioned area. Second, electrophysiological recordings of this region revealed that the area around the lesion contained neurons responsive to somatic stimulation (Fig. 10E and G), and that receptive fields could be easily obtained for area 2 in anesthetized monkeys. Further, they indicate that the techniques we employ for postoperative closure allow for an excellent neocortical recovery with no spurious damage from the dura adhering to the cortex following closure of the craniotomy.

Figure 10: Digital images taken before (A) immediately after (C) and 6 months after (E and F) area 5 was ablated in a titi monkey. Figures to the right represent the maps constructed from recording sites (small dots in digital images) using multunit mapping techniques. These techniques allowed us to quickly survey the cortex, identify the boundaries of area 5, lesion the cortex, and re-map the cortex. These same techniques were used 6 months later to assess the cortex surrounding the lesion. The aspirated portion of area 5 (C) is very small (1 x 2 mm). Electrophysiological recordings that were made immediately after aspiration demonstrated that neurons were responsive within 50 microns of the lesioned edge. In cortex examined 6 months after the lesion, the large blood vessels from earlier mapping sessions could be identified; the cortex around the lesion was completely recovered, and neurons were responsive to somatic stimulation. The area of cortex that was aspirated appears as a small slit (F). The map in G looks lengthened in the caudal direction because the recording sites at the edge of the lesion were into the depths of cortex. Cortex adjacent to area 5 (area 2) curled around the rostral edge of the lesion giving the appearance of a sulcus.

The brain from this experiment was sectioned and stained for Nissl, myelin and cytochrome oxidase (Fig. 8B and C). Examination of the cortex on the side of the lesion reveals that areas 3b and 1/2 can be identified, and that the extent of the lesion is the same size as that estimated from the digital images and electrophysiological recordings made immediately following aspiration of area 5 (Fig. 8B and C). In other words, these techniques allow us to make a small, targeted lesion with little damage to adjacent cortex, and to immediately determine the full extent of the lesion, rather than waiting many months later, after the brain is cut.

Two of the three lesioned monkeys exhibited a shift in hand use that was observed immediately after recovery from surgery. Although, as noted above, one of these monkeys was stricken with the virus so that post-lesion behavioral observations were limited. The posture of the hand contralateral to the lesion site was abnormal, with the digits splayed open and extended, in contrast to the normal posture in which the digits form a very relaxed fist (Fig. 11A and D). Additionally, within the first postoperative week, the monkeys were unable to use the hand contralateral to the lesion to retrieve treats offered by the
experimenter. The animal would attempt to reach with the hand, but the grasp was typically unsuccessful, resulting in the animal extending the ipsilesional hand (ipsilateral to the lesion) to retrieve the treat and bring it to the mouth. (Fig. 11C and D). The deficit observed with the contralateral hand appeared to be restricted to goal-oriented reaching, since normal locomotor use of the hand was observed during the same time period (Fig. 12). The animal could use the hand to climb, and was able to support itself while hanging from the hand. After approximately one week, the animals still preferentially used the ipsilesional hand, even for targets presented to the contralateral side of the body. However, the animals did begin to use the contralateral hand more frequently and more successfully for visually guided reaches. One interesting observation was that the animal would frequently gaze at the contralateral hand prior to making a visually guided reach. We did not observe this behavior at any time prior to the lesion, or with the ipsilesional hand.

Figure 11: Image taken from video footage of a tita monkey with a lesion to area 5 in the left hemisphere. This animal showed a right hand preference. The video was made 3 days following lesion surgery, and the frames from this video demonstrate the grasping problems encountered by this monkey. A demonstrates the splayed hand posture of this monkey, B demonstrates the animal’s ability to reach for the object, but not grasp it. In C, the animal grasps the object with the ipsilesional hand and brings the object to its mouth (D).

Handedness in monkeys. Previous studies indicate that many primates have a hand preference, including adult macaque monkeys (e.g. Welles, 1976; Horster and Ettlinger, 1985; Lehman, 1978; 1980a; 1980b; 1980c; 1989), and our own preliminary work in tita monkeys indicate that 30% are handed in that they use one hand > 80% of the time. In the experiments proposed in this application, we will use only animals that are handed. We have gathered preliminary data on six macaque monkeys to determine if these monkeys were ambidextrous, or preferred to use one hand more than 80% of the time. The monkeys were given the option to use one or the other hand in a grasping task that required the animal to reach and grasp a treat located +/- 60°, 30° and 0° degrees in horizontal space. A handedness index (HI) was calculated as the number of trials using the preferred hand/ the number of trials tested, restricted to locations – 30°, 0° and + 30°. Our results indicate the 2 of the 6 monkeys showed a clear hand preference (HI > 0.80).

Pre-lesion behavioral results in macaque monkeys. Currently, we have examined the behavior of two normal monkeys for all three tasks outlined in our Research Design and Methods. These tasks include:

Task 1. Visually guided and non-visually guided reaching and grasping
Task 2. Bilateral coordination of the hands
Task 3. Interhemispheric transfer of object shape

The first task requires the monkeys to reach under either visually guided or non-Visually guided conditions to one of 5 horizontal wells to retrieve a treat. The monkey learned the visually guided task with no training, and was able to perform the non-visually guided task in three sessions (50 trials each). We are currently collecting data on this group of experiments. The second task is much more difficult. The monkey is required to reach into a box with both hands, pull a cylinder upward with one hand, and retrieve a treat with the other hand (Fig. 14). This task requires the animal to use both hands in a coordinated fashion to retrieve a
treat. The box construction with electronics is complete and we have been collecting data for 2 months. One normal monkey began performing task 2 in both visual and non-visual conditions within five daily sessions of approximately 50 trials each. A second normal monkey has also been trained to use the task 2 apparatus, and was able to perform the task in both visual and non-visual conditions within ten daily sessions of approximately 50 trials each. We have since increased the length of training sessions to approximately 120 trials for each monkey, since both monkeys are performing the task for 98% of the trials in both conditions.

13. Histogram of preliminary results in one normal macaque monkey. Retrieval latency was significantly shorter for the left reward location of the food crevice (p<.01), compared to center and right reward locations, regardless of visual condition. No significant difference was observed between center and right locations. Retrieval latency in the visual center reward location was significantly shorter than the non-visual center reward location (p<.01). No significant difference was observed between visual and non-visual retrievals in the left or right reward location. Note the tendency towards increased variance in the non-visual condition relative to visual conditions.

Figure 14. A comparison of visual (left) and non-visual trials (right) for Task 2. When the green LED is illuminated, the monkey reaches into the apparatus with one hand (A and D). The monkey then brings the other hand into the box and uses both hands in a coordinated manner to lift the elevator (B and E), and retrieve treat with one hand while holding the elevator with other hand (C and F). The hand use is almost identical in the visual and non-visual condition. Although the monkey initially lifts the cylinder with the right hand, he uses the left hand to hold it up while he retrieves the reward with the right hand. These trials were obtained within the same testing session. Arrows indicate the food reward. In the visually guided condition, note the animal’s facial expressions. This is a beautiful example, in a natural situation, of the strong interaction between the visual system, and the use of the hands for accomplishing a task.

In our initial analysis of performance data for the one normal macaque monkey, one or more differences were observed among the reward positions F(2,363) = 34.41, p < .01. The Tukey multiple comparison test found that the retrieval latency for the left reward position (M = 4.68, SE = 0.22) was significantly shorter than the latencies for both the center (M = 6.60, SE = 0.24) and right (M = 7.21, SE = 0.23) Fig. 13). However, no difference was found between the center (M = 6.60, SE = 0.24) and right reward position (M = 7.21, SE = 0.23) reward positions (p > .05). Simple-effects test revealed that retrieval latency for the visual condition was significantly shorter (M = 5.69, SE = 0.34) than the non-visual retrieval latency (M = 7.50, SE = 0.34) for the center reward position, F(1,363) = 14.65, p < .01. No differences in latency between visual and nonvisual conditions for the left F(1,363) = 0.06, p > .05, and right F(1,363) = 0.08, p > .05, locations were observed.

The decreased latency on the left well location is likely explained by the stereotypic strategy employed by this monkey. In all trials, this monkey lifted the reward cylinder with the right hand. For the left location, this resulted in the monkey simply plucking the reward with the left hand. However, for the right location, the monkey typically lifted the cylinder with the right hand, switched hands holding the cylinder, and then retrieved the reward with the right hand (Fig. 14), resulting in an increased latency.
relative to the right location (Fig. 13). For the center location, the monkey lifted the cylinder with the right hand and retrieved the reward with the left hand, but the center location required a more complex hand configuration than the simple pluck used for the left locations, so that the latency was increased relative to the left location. One possible explanation for the lack of significance between visual and non-visual conditions in the right and left wells is that a relatively simple plucking motion of the retrieval hand can be used for these locations. Because the motion is fairly simple, visual input does not result in any significant latency change to the retrieval. In the center treat location, however, the monkey must change the configuration of his retrieval hand (i.e. a simple pluck is not used) in order to grasp the reward. Visual guidance of the retrieval hand appears to decrease the latency required in the center location, as compared to the non-visual condition in which the monkey can only use tactile cues to guide the retrieval.

For the third task, we have finalized construction of the box in which we will examine interhemispheric transfer of information across hemispheres. In this task the animal is required to discriminate an object of a particular shape with the one hand, while choosing the identical shape (from a selection of 2-3 shapes) with the opposite hand to retrieve a treat. We have successfully trained the animal to perform this task under visual guidance (Fig 15). After three preliminary sessions of approximates fifty trials each, the monkeys were able to successfully reach the correct target after presentation of the sample shape. The monkeys are currently training with the three possible sample/shape configurations, and the non-visual condition of training will be added one the target shape training is complete (expected within ten session). The electronics portion of this apparatus is currently being assembled.

D. Research Design and Methods

I. Multunit electrophysiological mapping: Placement of injections of anatomical tracers and lesions, and assessing cortical plasticity in area 2

While previous investigations have demonstrated that neurons in area 5 respond optimally when the animal is awake and performing a particular task (e.g. Mountcastle et al., 1975), our preliminary results indicate that neurons in area 5 can be driven in monkeys anesthetized with low levels of isoflurane (1 – 1.5%). This anesthetic has not been used in previous mapping studies of area 5 in monkeys, and as noted above, the level of isoflurane is directly related to activations in cortex (responsiveness of neurons) on the postcentral gyrus of macaque monkeys. The use of multunit recording techniques offers several important technical advantages for the types of studies we propose here, compared to single unit techniques in the awake animal. First, we can survey a very large region of cortex in a very short period of time by recording from over a hundred different locations in a period of a few hours.

This is critical for defining the body part representation to be injected for neuroanatomical studies (see below) and identifying the boundaries of an entire field, such as area 5, to be lesioned. This technique also allows us to rapidly determine the full extent of our lesion immediately following aspiration, before the craniotomy is closed and the animal is recoverd, rather than waiting until the end of our post-lesion behavioral experiments and our acute recording experiments. Second, multunit recording techniques do not require the use of a guide tube, or the implantation of a chronic recording chamber. Both of these techniques can generate a good deal of cortical damage, particularly after extended chronic recording. This makes the use of an awake, single unit recording techniques less than ideal when combined with studies of neuroanatomical connections, and lesion studies. Third, by using multunit recording techniques in an acute preparation, we can survey a very large region of cortex (e.g. areas 1, 2 and 5), and generate high density maps which will allow us to assess one important aspect of cortical organization that we propose will be modified in our lesion experiments, the magnification of a particular body part relative to other body part representations (for area 2 plasticity studies).
Missing from original grant application package.
anatomical plasticity in area 2, we can easily record from other cortical fields such as lateral sulcus areas S2 and PV (e.g. Krubitzer et al., 1995), or the contralateral area 5. Further, for our neuroanatomical experiments, we can place injections into other cortical fields and thalamic nuclei. However, we believe area 2 is a good candidate for plasticity because it receives proprioceptive inputs, has dense connections with area 5, and has callosal connections of the hand representation. These features indicate that area 2 is part of the cortical network associated with proprioception and visually guided reaching and grasping, and may take over some of the functions of area 5. In addition, this will be the first feed-back cortical plasticity study, and any result will contribute significantly to our understanding of cortical plasticity mechanisms.

II. Neuroanatomical studies: Although connections of area 5 have been described previously in macaque monkeys (see Background and Significance), the injection sites were extremely large and encompassed several areas in addition to area 5, the injection sites were not defined electrophysiologically (except one case in one study, see Background and Significance), nor were the projection targets related to electrophysiologically defined cortical areas. Further, previous studies examining area 2 connections utilized different tracers which produced varying sized injections, and no measurements of injection volumes were made. Therefore, these studies cannot serve as appropriate controls for the present investigation. By examining the connections of areas 5 and 2 we can appreciate the normal cortical and subcortical connections of these fields, as well any change in the connections of area 2 resulting from our lesions. Such changes in connectivity may contribute to the behavioral deficits we expect to see.

Figure 16. A schematic of the first type of neuroanatomical experiment. In these experiments, the same body part representation will be injected in different fields and the resulting patterns of connections with electrophysiologically identified body part representations in other fields in the same hemisphere or contralateral hemisphere (as shown here) will be examined. We propose that these types of connections between hand and forelimb representations across hemispheres form the substrate for bimanual coordination in primates.

These experiments will be of three types. In the first type of experiment, multiunit electrophysiological recordings will be used to identify the same body part representation in areas 2 and 5 so that connections of these fields in normal animals can be directly compared (Fig. 16). In the second type of experiment, different body part representations in the same field will be injected with neuroanatomical tracers into electrophysiologically identified locations so that details of topographic interactions between cortical fields in normal animals can be assessed (Fig. 17). In the third type of experiment, different body part representations in electrophysiologically identified locations in area 2 will be injected in post-lesioned animals. Injections will be made in area 2 between the two probes placed during the lesioning portion of the experiment (Fig. 3; see limitations below). In these latter experiments, injections in area 2 following lesions in area 5 and post lesion behavioral training will be matched to the body part representation injected in normal animals. Further, the same tracer will be used for the same body part representation in both normal and post-lesion animals. In all three types of experiments, cortical fields interconnected with the injected field will be explored using electrophysiological recording techniques after the appropriate time for transport of tracer.

For all experiments, small amounts of the neuroanatomical tracers fluororuby (FR), fluoroemerald (FE), and biotinylated dextran amine (BDA) will be injected into electrophysiologically identified locations of areas 5 and 2. These tracers consistently reveal both retrograde and anterograde label, the effective uptake zone is small compared to other tracers, the amount of necrosis resulting from injections is minimal and they have the same rate of transport. After the appropriate survival time, the animal will undergo an acute electrophysiological mapping experiment (which may last up to 2-3 days), in which some of the ipsilateral target fields (e.g. areas 1 and 5 for normal area 2 injection) and contralateral target fields (e.g. areas 5 and 2 for normal areas 5 and 2 injections) will be identified (Figs. 16 and 17). In all cases, the volume of each injection will be measured by generating a 3D reconstruction of the sites using Amira software and associated hardware (e.g. Fig. 6). Injection volumes will be calculated as a percentage of the entire cortical area injected as defined electrophysiologically and architectonically, as well as a percentage of the entire cortex. This will allow direct comparisons of the volumes of injection sites to be made and related to the presence or absence of connections within a cortical field or thalamic nucleus. In addition, measures of cell and terminal bouton density within a particular target cortical area or thalamic nucleus can be directly compared for injections of the same volume within and across cases. Data obtained in all neuroanatomical and electrophysiological experiments will be related to architectonic boundaries determined from histologically processed brains (see Methods). We will also generate a "flattened cortex" reconstruction of our data using the freely available CARET software generated by the Van Essen Lab. This will allow us to appreciate the overall pattern of transported tracer with respect to major sulci, other cortical fields, and topographic maps within cortical fields (as define electrophysiologically (Fig. 19).
While similar concentrations and volumes of anatomical tracer will be placed into the same electrophysiologically identified body part representation in normal and post-lesioned animals, this does not ensure that injections in both groups will be of the same size. Therefore we will do a post-hoc analysis of injection volumes, and compare only those in which volumes are similar, and which the spread into body part representations is the same (see Fig. 6). These combined neuroanatomical and electrophysiological experiments described here will allow us to assess several features of connectivity (Figs. 16 and 17) which may be related to the types of behavioral recovery we expect to see. For example, in normal animals these include:

1. The amount of convergence from different body part representations in area 1 onto particular body part representations in area 2 (e.g. Fig. 17)
2. The amount of convergence from different body part representations in area 2 onto particular body part representations in area 5
3. The amount of divergence of projections from area 2 to different body part representation in area 5 (e.g. Fig. 17)
4. The amount of divergence and convergence in callosal connections of area 2 and 5
5. The amount of divergence and convergence in thalamic connections of areas 5 and 2

In post-lesioned animals they will allow us to determine:

1. If new connections between area 2 and other cortical fields have formed. Regardless of the injection volume, the presence of new connections (e.g. between area 2 and PRR or SMA; Fig. 3) will unequivocally demonstrate that anatomical plasticity has occurred.
2. If connections between area 2 and other cortical fields have dramatically increased in density. We believe our techniques will allow us to determine if extreme density differences in labeled cells or terminals exist between the normal and lesioned animals, although subtle differences in density are unlikely to be detected with our techniques.
3. If new projections from different body parts in particular fields (e.g. area 1) have emerged (Fig. 3).

**Figure 17.** A schematic of the second type of neuroanatomical experiment. In these experiments, different body part representations in the same cortical field (e.g. area 2) will be made, and the resulting patterns of connections with electrophysiologically identified body part representations in other fields in the same hemisphere (as shown here) or contralateral hemisphere will be examined. These types of experiments allow us to determine the amount of divergence and converge in connectivity that exists for any particular cortical field. In this example, the hand representation in area 2 had convergent connections from the shoulder, trunk, hand and face representations in area 1 and divergent projections to area 5. These experiments allow us to speculate on the anatomical substrate for normal manual and bimanual behavior as well as to explore potential anatomical plasticity after behavior recovery from lesions in area 5.

**Expected outcomes and Limitations:** The data on connections of area 2 in normal animals will be used as controls for the lesioned animals. There are two potential outcomes. First, there may be no observable differences in connections of area 2 between normal and lesioned animals. This would indicate that any behavioral recovery observed in these animals is due to small synaptic changes (Fig 2) that are not discernible with our methods, or that the site of change is not area 2. A second potential outcome is that there will be modifications in the connections of area 2 in lesioned animals manifested as 1) an increase in the density of labeled terminals or cells in different areas compared to normal animals, or 2) the presence of new projections to novel cortical areas in lesioned animals compared to normal animals. There are a few studies which demonstrate that changes in connections can occur in adults with peripheral deafferentation (Florence et al., 1998), retinal lesions (Darian-Smith and Gilbert, 1994), or lesions in M1 (Dancouse et al., 2002). In these studies, new horizontal connections formed within a cortical field, which would be indicated in our experiments as an increase in amount the of label as a function of the injection site. However, there is also evidence that axonal sprouting occurs to novel cortical areas (e.g. Dancouse et al., 2002; R.J. Nudo, personal communication). Thus, while one may have previously thought that changes in anatomical connections would be unlikely, there is a distinct possibility that we will see changes in connections of area 2. To explore this possibility, the entire cortical hemisphere will be processed in each monkey to ensure that such new connections are identified. Because no new animals need to be generated for this portion of the experiment, and since there is some indication from previous work that new connections may form, we felt it was worth pursuing this issue in our already lesioned animals. If our result proves positive, it will profoundly alter the way we think about recovery of function following cortical lesions. Even in the event that no such changes are observed, or that the
changes are very limited in scope, such a result is still valuable in that it will limit the scope of interpretations of how behavioral recovery occurs in the lesioned animals (see below).

A final potential problem with these experiments has to do with the placement of our injections into electrophysiologically identified locations in area 2 in post-lesioned animals. Because we predict that the representation of the hand in area 2 will expand, it will be difficult to ensure that the representation of digit 2 in the post-lesioned animal, for example, is actually analogous to the representation of digit 2 in normal animals, rather than in the location of what would normally be digit 3. To circumvent this problem, we will place very small, plastic probes in area 2 during the lesion portion of the experiment, which can be visualized many months later during the acute mapping phase of these experiments. Surgical placement of plastic probes and subsequent visualization of these probes in acute mapping experiments (over 1 year) has been done previously in our laboratory. High density recordings will be made between the two probes and receptive fields for portions of the hand and digits obtained. Upon re-opening, the probes will be visualized, the cortical surface imaged (see Methods), and cortex between the probes will be explored in detail to determine if cortical map changes have occurred. If they had, then the site of injection will be made between the two probes at a location pre-determined in the lesion phase of the experiments (e.g. at the representation of digit 2 in pre-lesion maps).

III. Behavioral experiments. We will train three groups of monkeys on three different tasks (one task for each group) that will allow us to assess the effects of area 5 lesions on particular manual behaviors. Each of these groups will also be trained on a motor task and texture discrimination task to allow us to assess some of the motor and tactile abilities that are spared. For all trained tasks, the animal will be placed in a primate containment-housing device (SDIA products) to restrain head movements and gross body movements. These chairs allow the animal to move its arms and hands freely to perform natural reaching and grasping movements. All monkeys for all trials will be videotaped. In addition, feeding sessions pre and post lesions will be videotaped, as well as one 10 minute session per day in which the experimenter hands the monkey objects and food to grasp and manipulate.

Determination of handedness. Prior to the commencement of these studies we will assess the strength of handedness of monkeys from the large group available at the California National Primate Research Center (CNPRC). A handedness index will be derived by calculating the number of trials using the contralesional hand divided by the total number of trials for the three middle positions. Animals with a handedness index of ≥ 0.80 on this task will be accepted for further study. This screening procedure will also allow us to identify individual monkeys that are tractable to the task and are appropriately motivated to consistently perform such reaching tasks.

Starting posture, go cues, and timing mechanisms. For all three tasks, the monkey will be mildly restrained in a primate chair, and the behavioral apparatus will be placed within reach. The apparatus consists of two levers, a green LED at eye level, and a test box that the monkey can access with one or both hands through one or two openings in order to retrieve a food reward. The timing of events during each trial will be measured by a personal computer with associated I/O cards (Access I/O Products, Inc., San Diego, CA). The monkey will initiate each trial by depressing both of the two levers with either hand, monitored via microswitches. The investigator will move the front panel (see below) and following a randomized delay of 500 – 1500 ms, the LED will be illuminated signaling the monkey to make a response. For all tasks, the monkey must place one or both hands into the test box. Entrance and exit of the hand and arm into the test box is measured by the opening and closing of a door at each opening. Thus, accurate measurements will be recorded for 1) the hand and latency of the initial movement, 2) the time for the hand to enter the test box, and 3) the time the hand(s) spend(s) in the test box.

Group 1 – visually guided and non-visually guided reaching and grasping. These tasks require the animals to retrieve a reward from 1 of 5 horizontally displaced locations under visually guided and non visually guided conditions. In these experiments, the test box will contain 5 wells placed at equidistant locations at 0°, +/− 15° and +/− 30° in azimuth. The floor of the box will be marked with a 1 cm grid pattern to quantify any targeting errors using our videos. The investigator will slide the front panel of the box to one of two positions. The first will be transparent with a hinged door that the monkey can reach through to obtain the reward. This configuration is used in the visually-guided reaching task. The second is made of opaque gray plastic, including the hinged door at the opening. This configuration will prevent the monkey from seeing the reward during the trial and will be used in the non-visual guided condition (as shown in Fig 14 for Group 2 experiments).

For each trial, the reward is placed into one location. The monkey must then grasp both handles, and the investigator will move the door to either the visually guided or non-visually guided position. Once the LED is illuminated, the monkey will remove the hand from the lever, insert its hand into the test box, retrieve the reward and eat it. For all trials, the monkey must initiate a reach within 15 seconds of the start of the trial, or a "balk" will be recorded. If the monkey has initiated a reach but does not recover the reward within 15 seconds, the trial will be recorded as "fail." The location of the reward and whether the trial is visually or non-visual guided will be randomly selected before each experiment as five sequences of ten trials. Each location and condition will therefore be presented in each sequence before the next sequence is started. In this way there will be no more than 1 trial difference between trial types if the monkey stops working early. The primate containment chair allows us to keep one arm of the monkey immobile by blocking access to the box. This will allow us to examine the monkey's performance using either the
contralateral or ipsilateral hand. Four different trial conditions include Vis/Vis, contralateral hand, Vis/Non-Vis, contralateral hand, Vis/Vis, ipsilateral hand and Vis/Non-Vis, ipsilateral hand. The accuracy and precision of the grasping movements will be measured by post-hoc analysis of the video tape using the cm grid as a reference. Accuracy will be measured as the mean error to the target, while precision will be measured as the standard deviation of the mean error.

Group 2 - bilateral coordination of the hands: This task requires the animal to use both hands in a coordinated fashion to lift a small cylinder to retrieve a reward pellet. For this task, the front of the box will contain two openings with hinged doors such that both hands can be inserted into the test box simultaneously. The monkey will be trained to place both hands on levers mounted lateral to the doors of the testing box, in response to blinking red LEDs mounted above each lever. Once both hands are depressing the levers, the red LEDs will turn off, and a centrally mounted green LED will turn on, indicating the start of the trial. The monkey will then release the levers, and reach into the box through the doors in order to grasp and lift the cylinder. One hand must be used to lift and hold the cylinder, while the other retrieves the pellet (Fig. 14). The monkey will be trained to immediately remove both hands from the box after retrieving the pellet, and await the next trial sequence. The apparatus is constructed to allow us to vary the placement of the pellet holder to the left, center, or right side of the cylinder. Trials will be presented visually and non-visually by means of a small screen that can be moved into place to block the window prior to the start of selected trials (Fig. 14). Six different trial conditions are possible using all configurations of the apparatus (Vis/position 1, Vis/position 2, Vis/position 3, Non-Vis/position 1, Non-Vis/position 2, Non-Vis/position 3).

During each testing session, blocks of five trials per condition will be presented, with the order of the blocks assigned randomly. For example, a block of five trials of visual condition with pellet on the left would be followed by a block of five trials of non-visual condition with pellet in the center, etc. Blocks will be continued until each condition has been presented for a minimum of ten trials per condition per session. Timing data for every trial will be collected as latency from green LED on to 1) lifting levers, 2) opening doors, 3) lifting cylinder, 4) replacing cylinder, and 5) closing doors.

Group 3 - interhemispheric transfer of object shape: This task requires the animal to use visual and haptic cues or haptic cues alone to sample an object of a particular shape with one hand, while choosing the identically shaped object with the other hand, from an array of different shaped objects. The apparatus for this experiment will have two openings, one for each hand, that will allow access to two different compartments (Fig. 15). The investigator will place one object (the sample) in the compartment corresponding to either the contralateral or ipsilateral hand. A similarly shaped object (the target) will be placed in the other compartment, as well as one or two differently shaped objects (distracters). The monkey will be trained to place both hands on levers mounted lateral to the doors of the testing box, in response to blinking red LEDs mounted above each lever. Once both hands are depressing the levers, the red LEDs will turn off, and a centrally mounted green LED will turn on, indicating the start of the trial. The monkey will then release the levers, and reach with both hands into the box through the doors. The monkey will be trained to grasp the sample shape presented singly on one side of the box, and then grasp the matching shape (target) on the other side of the box from an array of 2-3 objects, using the opposite hand. Upon grasping the matching shape, the monkey will pull the shape forward approximately two centimeters in order to uncover a reward pellet and retrieve it using the same hand (Fig. 15). If the monkey pulls the incorrect shape forward, the trail will end and the monkey will not receive a reward. The monkey will be trained to remove both hands from the box immediately upon retrieval of the pellet, to await the next trial.

Two moveable opaque screens mounted on the front wall of the apparatus allow us to present the sample shape and target shapes visually or non-visual, resulting in two conditions for both hands (V/V, N/N), and four different visual conditions for the contralateral and ipsilateral hand (sample/targets: NV, VN; target/sample NV/VN – see Figure 18). The NV condition for the sample ipsilateral hand indicates that the monkey cannot see the sample but can see the targets. The VN condition for the sample ipsilateral hand indicates that the monkey can see the sample hand but cannot see the targets. The NV for the ipsilateral target hand indicates that the monkey cannot see the sample but can see the target. The VN for the ipsilateral target hand indicates that the monkey can see the sample but cannot see the targets. These same conditions hold for the contralateral hand (Fig. 18).

The objects will be in the easily discriminated shapes (tetrahedron, 4 sided; hexahedron, 6 sided; icosahedron 20 sided). Either one or two distracters will be presented on randomly interleaved trials. The location of the matching target object will be varied randomly. Timing data for every trial will be collected as latency from green LED on to 1) lifting levers, 2) opening doors, 3) grasping sample object, 4) grasping matching object, and 5) closing doors. Monkeys will initially be trained on the easiest discriminations under the all-visual condition, and the complexity of the task will gradually increase as the monkey's performance improves.

Examination of spared function: In order to determine whether the lesion induced a deficit in intended motor sequences associated with reaching, grasping and information transfer across hemispheres, rather than a pure motor or somatic deficit, each of the above three groups of animals will be trained on 2 additional tasks.
The act of grasping (motor) Our preliminary studies in titi monkeys indicate that immediately following the lesion, the animals do not use the contralesional hand, but they can navigate in their cages and use this hand to grasp the bars during locomotion and stabilization of the body (Fig. 12). Thus, it appears that the ability to grasp is not impaired (i.e. the motor sequence for grasping appears normal). Rather, the animal cannot intentionally act upon a desired object with the hand. To test the motor grasping abilities that have been spared, the animal’s cage will be fitted with a ladder with rungs of a size that requires the animals to grasp them with each hand to climb to the top of the ladder. A desired treat will be placed at the top of the ladder, and the animals will be required to climb the ladder to obtain a reward. This will ensure that the grasping deficit induced by the lesion is not due to an inability to make the appropriate motor movement of the hand and arm.

Somatic discrimination Previous studies in which the primary somatosensory area was lesioned indicate that the animals have lost the ability to make texture discriminations, an ability proposed to be generated by areas 3b and 1 (see Background and Significance). In order to ensure that the lesion is specific to goal-directed grasping and not to an inability to detect somatic stimuli with the contralateral hand, a simple texture discrimination will be performed. This task is similar to that of task 1 in which the monkey must reach into a test box. Within the box are two different textured panels covering a treat well. Under non-visually guided conditions, the monkey will be trained that the treat is under the “soft” texture (cloth) and not the “rough” texture (sandpaper) and must rest its hand over the soft texture to receive the reward (which will be hand fed to the post lesion animals).

Expected outcomes and limitations: For each group we will refer to the hand ipsilateral to the lesion as the ‘ipsilesional’ hand and the hand contralateral to the lesion as the ‘contralesional hand’. Our experience with the titi monkeys indicates that initially these monkeys may not be able to perform any of the tasks in Groups 1-3, but should gradually recover at least some function over the course of the next several weeks. For this reason, the first post-lesion testing day (likely to be 2-4 days post-surgery) will start with the simplest versions of each task (e.g. visually guided).

Group 1 Prior to the lesion, we expect these monkeys to use (almost exclusively) their contralesional hand to perform the task. However, when forced, they will be able to perform this task with the ipsilesional hand. We expect short latencies for time in the box and low error rates, particularly under the visually-guided condition. We expect greater accuracy and precision for the visually-guided task compared to the non-visually guided task.

Following the lesion, we expect the animal to be able to perform this task under visual and non-visual conditions with the ipsilesional hand, but not with the contralesional hand. In each session, we will attempt to ‘force’ the monkey to use the contralesional hand to perform the task by either: 1) moving the orientation lever for the contralesional hand farther away from the opening in the apparatus, thus making the reach with the contralesional hand much easier than with the ipsilesional hand, and/or 2) having the hinged door ‘locked’ if the ipsilesional hand is used. Data from titi monkeys indicates that there should be some behavioral recovery for this task over time, and therefore we expect that encouraging the monkey to use the contralesional hand will prove effective. Over the course of several days to weeks, the monkey should begin to use the contralesional hand, and will begin to perform this task under visual guidance, although with decreased accuracy and with increased latencies. Both of these metrics are expected to improve, and eventually the performance will become similar to (and perhaps indistinguishable from) pre-lesion performance. One potential drawback, particularly early after the lesion, is that the monkeys will simply not attempt to initiate a trial, or be unable to effectively grasp the orientation handle with the contralesional hand. Relaxing this criterion will reduce this problem, and ‘forcing’ the monkey to use the contralesional hand on at least some trials will indicate how performance is improved over the course of the first several days to weeks after the lesion.

Group 2 Prior to the lesion, we expect monkeys to perform this task under visual and non-visual conditions, with longer latencies for the non-visual condition, at least for some crevice configurations. Following the area 5 lesion, we predict that initially the animals will attempt to perform this task with the ipsilesional hand alone under visually guided conditions. With recovery, the animal will begin to use the contralesional hand. At first we expect the hand to be used awkwardly, with longer latencies in the box. As time progresses, we expect the time in the box to decrease, but to be different depending on the initial orientation of the reward cylinder. All of these expectations are for the visually guided condition. It is possible that the animal may never be able to perform this task without visual guidance. Studies of interhemispheric transfer after sections to the corpus callosum indicate that monkeys cannot perform transfer tasks without visual guidance (Manzoni et al., 1973). However, since there are interhemispheric connections between the hand representations of cortical areas 2, S2, PV (see Dibbrow et al., 2001a; 2003a), recovery may be possible. It is also possible that new interhemispheric connections may form, and that these connections may allow for some recovery of function under non-visually guided conditions.

Group 3 Prior to the lesion, we expect that the monkeys will perform this task using both the contralesional and ipsilesional hand as the sample hand, under both visual and non-visual conditions, although non-visual conditions will have longer latencies. Following the lesion we expect that the monkey will be unable to perform this task using either hand as the sample hand under non-visual or visual conditions. We expect that the animal will ultimately regain the ability to perform this task under full visual guidance, and in some visual guidance conditions with both hands as the sample hand (see Fig. 18), but will never regain the ability to perform this task under complete non-visual conditions.
A decrease in the accuracy and/or precision of reaching movements in the ipsilesional hand will indicate that unilateral lesions of area 5 have a bimanual influence. This is not wholly surprising since there are large, bilateral receptive fields for neurons in area 5, indicating cross-hemispheric processing by this area. Comparisons between the all-visual and non-visual conditions should reveal a large deficit in the ability of the monkey to transfer the shape of the object from the lesioned hemisphere to the unlesioned hemisphere.

An alternative finding for all three tasks is that there is little if any deficit as a result of these lesions. This seems unlikely given the preliminary data from tiki monkeys and previous lesion work in monkeys and humans, as well as the fact that interhemispheric connections between representations of the hand are limited to only a few somatosensory fields, one of which is area 5. However, as noted above, spared connections of lateral somatosensory fields may allow the animal to perform these tasks. This would indicate that these lateral fields are sufficient for interhemispheric transference of shape information.

<table>
<thead>
<tr>
<th></th>
<th>Ipsilesional Hand</th>
<th>Contralateral Hand</th>
<th><strong>Expected Outcomes and Interpretation</strong></th>
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<tbody>
<tr>
<td><strong>Condition A</strong></td>
<td>visual</td>
<td>non-visual</td>
<td>- Can't perform the task - Area 5 is necessary for shape discrimination</td>
</tr>
<tr>
<td><strong>Condition B</strong></td>
<td>non-visual</td>
<td>visual</td>
<td>- Can perform the task - Other fields are performing shape discrimination in addition to area 5, or instead of area 5</td>
</tr>
<tr>
<td><strong>Condition C</strong></td>
<td>visual</td>
<td>non-visual</td>
<td>- Can't perform the task - Area 5 is necessary to receive intermanual transfer of shape information across hemispheres</td>
</tr>
<tr>
<td><strong>Condition D</strong></td>
<td>non-visual</td>
<td>visual</td>
<td>- Can perform the task - Information regarding shape is transferred across hemispheres in areas other than area 5, or in addition to area 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- If the monkey can perform condition A experiments but can't perform condition C experiments, then area 5 is not necessary for shape discrimination, but is necessary for the transfer of shape information to the other hemisphere</td>
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**Motor and somatic discrimination**

Prior to the lesion, we predict that the monkeys will perform the ladder task with ease. We also predict that the monkeys will perform the texture task with minimal training. Immediately following the lesion, it is likely that monkeys will show no deficits at either of these tasks. Designing a tactile discrimination task that does not possess an intended reach is difficult. However, our results in tiki monkeys indicate that although the hand is initially splayed and the animal cannot grasp intended objects, it can reach to these objects. Studies in macaque monkeys in which large lesions were made to posterior parietal cortex that incorporated area 5 indicate that the range, velocity and hand trajectory was not affected (Rushworth, 1997). Thus, the animal should be able to reach into the box and contact the different textures with its splayed hand. We believe texture discrimination will be spared since lesions will not incorporate anterior parietal fields involved in this type of discrimination (Kandolph and Semmes, 1974; Carlson, 1981). If the monkey can perform this task, it will be hand fed the treat by the investigator, since it will not be able to grasp the treat. The second issue regards the motor task and the ability to climb towards a treat. We believe that the animal will be able to climb the ladder in a stereotypical fashion, as do tiki monkeys, but not be able to grasp the treat at the top. If this is the case, as in the somatic discrimination condition, the animal will be hand fed the treat by the investigator. If the animal cannot perform this task, the 1/2 hour morning and afternoon video sessions (see above) will be examined and the use of the hand in grasping during locomotion and non intended grasping conditions will be determined.

**IV. Lesion experiments** Once animals have reached a criterion (no significant change in at least 10 consecutive training sessions), area 5 contralateral to the preferred hand will be lesioned and each group of animals will be tested for 3 - 6 months following the lesion (or until behavioral recovery asymptotes) on the tasks described above (see Methods below for details).

**Methods**

**1. Electrophysiological recording** At the beginning of these experiments, the animal will be anesthetized with ketamine hydrochloride(10 mg/kg). Once anesthetized, the animals will be cannulated and intubated, and given a continuous infusion of 2.5% dextrose in lactated Ringers, alternated with lactated Ringers alone. Inhalation anesthesia will be administered (1-1.5% isoflurane), and the animal will be artificially ventilated, and blood gas levels will be monitored and maintained at PCO2 between 35 and 45 mmHg, and PO2 at approximately 400 mmHg. Temperature, heart rate, blood oxygenation levels and fluid intake and output will be monitored in all experiments. After the animal is anesthetized, it will be placed in a stereotaxic frame. The skin
will be cut, the muscle gently deflected from the bone, and the bone over the IPS will be removed. The dura will then be cut, and the cortex exposed. For acute mapping experiments, an acrylic well will be built around the opening and secured to the skull with small screws. The well will then be filled with silicone fluid to help maintain cortical temperature and prevent desiccation. Tungsten electrodes (5MSΩ at 1kHz with tip exposures of up to 30µm), designed to record extracellularly from neural clusters and single neurons, will be used. Neural activity will be amplified, filtered and then heard through a loudspeaker and visualized on an oscilloscope. A number of closely spaced recording sites will be made and marked on an enlarged digital image of the brain, and receptive field location and stimulus preference obtained. In these types of experiments, it is possible to obtain over 1000 recording sites in a single animal, so that a large region of cortex can be surveyed. Small electrolytic lesions (10 µA for 6 seconds) will be made at estimated boundaries in acute mapping experiments. After the acute mapping studies are complete (these studies end when the animal expires, or when neurons in the cortex and/or thalamus cease to respond (~40 - 55 hours), the animals will be euthanized and transcardially perfused.

**Somatosensory Stimuli** Somatosensory stimulation of cutaneous receptors will consist of lightly touching or brushing the skin or deflecting hairs. Stimulation of deep receptors includes light pressure, lightly tapping the skin, or manipulating joints. These types of stimuli have proven effective for determining the extent of the cortex devoted to a particular sensory system, and for determining the boundaries of cortical fields within a given sensory system in previous mapping studies in primates (e.g. Krubitzer and Kaas, 1990; Huffman and Krubitzer, 2001a; Krubitzer et al., 2003). Von Frey hairs will also be used to determine the threshold of neurons in areas 2 and 5.

**Visual Stimuli** Because our goal is to determine only if neurons are responsive to visual stimulation, not the details of the stimulus required, or the response properties of neurons, we will use simple stimuli including backlit dark bars, circles of light, and full field flashes of light. These stimuli have been effective for stimulating neurons in area 5 in both tib and macaque monkeys.

**II. Injections of anatomical tracers** These experiments will be performed under standard surgical conditions in the sterile surgical suite at the CNPRC. Injections will be made under electrophysiological guidance using the methods described above. The region of interest including areas 5, 2 and 1 will be exposed and relatively dense electrode penetrations will be made in rostro-caudal rows beginning in area 1 and ending in area 5 (down the medial bank of the IPS Fig 10). Boundaries of fields will be determined by change in the subclass of receptors neurons respond to (e.g. cutaneous for area 1 and deep for area 2), reversal in receptive field progression, changes in receptive fields size, and changes in responsiveness (e.g. neurons respond well in area 2, and more weakly in area 5, neurons in area 2 are smaller than in area 5). Several rows of recording sites across the hand representation in areas 1, 2 and 5 will be made, and boundaries of the field will be estimated. Once the boundaries are determined, different body part locations within a field (e.g. forelimb vs. hand vs. face in area 2) will be injected, or the same body part in different fields (e.g. the digit -2 - 3 representation in area 2 and the hand representation in area 5) will be injected. After injections are complete, a soft sterile contact lens is place over the cortex, the dura is place over the lens, gelfoam is placed over the dura, and the craniotomy is closed with an acrylic cap. The procedures for injections of the different tracers, amounts, and survival times are as follows.

**Fluoroemerald (FE) and fluororuby (FR)** are two tracers to be used in these experiments. Both are transported retrogradely and anterogradely, and work maximally in soluble form when injected through a specially beveled Hamilton syringe. Approximately 0.3 - 0.5 µl of 7% BDA will be injected via a Hamilton syringe at the site of interest. BDA provides very detailed morphological labeling of cell bodies and axons (see Fig. 19), and is not subject to bleaching or fading over time (e.g. Reiner et al., 2000). The time course for transport of BDA is compatible with that of FE and FR. Additionally, the injection sites of FE and FR are visible in the BDA series of section, simply by switching to fluorescent viewing on the microscope. This allows direct comparisons of all injection sites to be made.

**Biotinylated dextran amine (BDA)** is an additional tracer that will be used for these studies. Approximately 0.3 - 0.5 µl of 7% BDA will be injected via a Hamilton syringe at the site of interest. BDA provides very detailed morphological labeling of cell bodies and axons (see Fig. 19), and is not subject to bleaching or fading over time (e.g. Reiner et al., 2000). The time course for transport of BDA is compatible with that of FE and FR. Additionally, the injection sites of FE and FR are visible in the BDA series of section, simply by switching to fluorescent viewing on the microscope. This allows direct comparisons of all injection sites to be made.

**Figure 19**: Injections of FE (A), FR (B), and BDA (C) into areas 5 (FR and FE) and AIP (C). Labeled cells from these injections in the ipsilateral cortex (D-F). Note that the injection sites are small, and little or no necrosis occurs. Scale bar for A-C is 500 microns. Scale bar for D, E is 50 microns. Scale bar for F is 100 microns.
After the injections are complete, a sterile soft contact lens will be placed over the cortex and the edges of the dura will be placed over the lens so that the dura does not adhere to the cortex during recovery. Upon reopening, we have found that the contact lens provides a permeability that mimics that of the dura mater, and a fluid layer of cerebrospinal fluid has formed between the contact lens and the cortex. After the lens is placed over the cortex, a piece of sterile gelfoam will be placed over this, the bone will be replaced and sealed with dental acrylic, the temporal muscle will be sutured, and finally, the fascia and skin will be sutured. After the appropriate recovery time has transpired, the animal will undergo acute electrophysiological recording so that some of the ipsilateral and contralateral targets of the injected area can be identified.

III. Lesion experiments. As with the neuroanatomical experiments, these experiments will be done in the sterile surgical suite at the CNPRC. Animal preparation, anesthesia and surgery will be like that described above for electrophysiological recording and anatomical tracing experiments. After the hand and forelimb representation of areas 2 and 5 are identified, two small plastic probes will be implanted at selected locations in the hand representation of area 2 for later identification in neuroanatomical experiments. Area 5 will then be aspirated, the cortex at the edges of the lesion will be explored electrophysiologically, receptive fields drawn, and neural preferences and strength of response noted. When this phase of the experiment is complete, the exposure will be closed exactly as that described for the neuroanatomical experiments. At the completion of post lesion behavioral testing (approximately 6 months following the lesion or when behavioral recovery asymptotes), the lesioned animals will undergo one of two experiments. 1) Electrophysiological recording of preserved cortex following the lesion (using methods described above) 2) Examination of connections of area 2 (using methods described above).

We chose to use aspiration as a method for lesioning the cortex, rather than ibotenic acid, because it allows us to have greater control over the size and spread of the lesion, and the time course of spread of lesion. The full effects of ibotenic acid are not realized until several hours, and up to several days following the actual injection (e.g. Hamada and DeLoog, 1992). As noted above, the size of the lesion and spread estimated immediately following aspiration conforms almost perfectly to the size of the lesion determined 6 months later using electrophysiological recording techniques and histological analysis. In two of our titi monkeys, the blood vessel distribution was such that very large blood vessels ran parallel to the IPS, much as in macaque monkeys. This did not create a problem with aspiration, as we were still able to remove the underlying cortex without compromising the vascular system.

Behavioral Data Analysis. The behavioral data for all three groups of trials will be scored primarily using latency to retrieve the reward. These data will be obtained from the contact circuits and microswitches on the primate chair and training boxes, respectively. In all trial groups, appropriate post-hoc analyses will be performed to determine the extent of any significant main effects and interactions among the factors analyzed with MANOVA. Additionally, the video records of all sessions will be used to qualitatively assess factors such as ability to grasp reward, and any changes in hand posture during the tasks.

Group 1 Trials: Quantitatively, a 2x5x2x2 repeated measure MANOVA (visual conditions; well locations; hand used, lesion status) will be performed for latency measures acquired throughout the task. Roy-Bargmann stepdown tests will then be utilized if a significant multivariate test is found. A 2x5x2x2 repeated measure ANOVA will be performed on the end-task latency measure if a non-significant multivariate test is found. A performance index will also be quantified to evaluate performance changes across pre- and post-lesion sessions. Performance Index = (successful trials - balked trials - failed trials) / (successful trials + balked trials + failed trials)

Group 2 Trials: A 2x3x2x2 repeated measure MANOVA (visual condition; reward position; hand used for retrieval, lesion status) will be performed for latency measures acquired throughout the task. Roy-Bargmann stepdown tests will then be utilized if a significant multivariate test is found. A 2x3x2x2 repeated measure ANOVA will be performed on the end-task latency measure if a non-significant multivariate test is found. Video recordings of Group 2 trials will be used to determine standard hand posture during grasp, and any changes in contralateral hand used during the trials.

Group 3 Trials: A 4x2x2x2 repeated measure MANOVA (visual condition, distracter number, hand used, for sample shape, and lesion status) will be performed for latency measures acquired throughout the task. Roy-Bargmann stepdown tests will then be utilized if a significant multivariate test is found. A 4x2x2x2 repeated measure ANOVA will be performed on the end-task latency measure if a non-significant multivariate test is found. The percentage of failures at each condition will also be calculated using the video record.

Histological processing of tissue. At the end of all experiments, all animals of all groups will be perfused with 0.09% PBS followed by 4% paraformaldehyde. Once excised from the skull, all brains will be cryoprotected in 30% SPB overnight. Fiducial probes will be inserted in a coronal plane at strategic locations in the brain to aid in 3D data reconstruction. Cortex will be sectioned horizontally at 80 μm on a freezing microtome, and block face images for every fourth section will be acquired for use in 3D data reconstruction. Alternate sections will be reacted for myelin (Gallyas, 1979), cytochrome oxidase (Carroll and Wong-Riley, 1984), Nissl, processed for BDA, or mounted for fluorescent microscopy. BDA will be visualized using standard
Data analysis. Data analysis is performed in stages and then all analyzed data are combined into a comprehensive reconstruction. First, the series of sections that are mounted for fluorescent microscopy or processed for BDA are analyzed using an X/Y stage encoding system (AccuStage, Shoreview, MN) attached to a Windows compatible computer. For the entire series of sections in each case, labeled cells, axon terminals and injection sites are plotted along with electrode tracks (when visible) tissue artifacts, section outlines, electrolytic lesions made during the electrophysiological recording stage of these experiments, and fiducial probes placed after the brain has been excised. Our plotting program allows us to count the number of cells within a selected X/Y area for any given section. Next, architectonic boundaries are added to these connections series of sections by matching blood vessels, fiducial probes and outlines of tissue from adjacent sections stained for Nissl, myelin and CO. Both cortical field boundaries and laminar boundaries within a cortical field will be determined. All data will be collapsed onto the previously acquired block face image and then either a 3D reconstruction of the brain will be made using the Amira software and hardware system (Fig. 6, see below), or a CARET flattened map will be generated. After this is done, recording sites on the dorsolateral surface of cortex (plus microlesions and probes) can be added by matching the digital image (which contains tracks, blood vessels and fiducial probes) to the 3D reconstruction. Electrophysiological maps of the brain are made by analyzing receptive fields and stimulus preference at all sites (taken from the digital image of the brain made prior to the start of mapping), and drawing lines which are interpolated between different body part representations. Reconstruction of our lesions can be done in the same manner. This type of reconstruction, although time consuming, allows us to combine all of our data sets with a great deal of accuracy. Some of these procedures have been described in detail elsewhere (Krubitzer and Callford, 1992; Krubitzer and Kaas, 1990; Krubitzer et al., 1998; Huffman et al., 2001a).

Recently we have acquired the Amira 3.0 software package (TGS, Inc; San Diego, CA) and a Dell 8250 workstation (Dell; Round Rock, TX). Together they serve as a state of the art image-processing tool that is specialized for very large, three-dimensional data sets. Amira can be used to import hundreds of digital images of serially sectioned and/or reconstructed (see above) neural tissue stained for various neuroanatomical markers, anatomical tracers, or reconstructed tissue containing lesions, X/Y stage encoded cell position information, and/or architectonic boundaries. Amira automatically aligns these images to generate a three-dimensional neural structure, and then labels various selected features within the reconstruction. The three-dimensional reconstruction may then be digitally "sliced" in standard or arbitrary planes of section, in order to best illustrate the features chosen by the user. This type of manipulation allows the user to visualize and measure nearly any perspective of the anatomical organization of the neural structures under investigation, and to determine the total volume of a lesion or injection site by calculating each as either an absolute volume or as a percentage of a selected region of the brain (e.g. forelimb representation of area 2) or the entire brain volume. We can also calculate the volume of a cortical field or body part representation within using this system.

We will also "flatten" the cortex to more readily compare overall patterns of connections with respect to the overall geographic relationships of cortical fields, sulci, and electrophysiological recordings generated from each experiment. This will be done using the freely available CARET software (Van Essen Laboratory, St. Louis), which we are currently utilizing in our laboratory. CARET is a software application kit designed for viewing and manipulating surface reconstructions of the cerebral and cerebellar cortex. It is used specifically for generating cortical flat maps, registering cortical maps to an atlas; and analyzing and visualizing many types of experimental data (e.g. neuroanatomical and functional imaging), gathered using a variety of techniques. Every type of experimental data we wish to analyze, and/or display, whether it is a topographic map, geographic landmarks or architectonic areas, connectivity patterns, or volume measurements can be projected onto a surface and stored in an easily accessible file. Information can then be viewed and/or edited. One of CARET'S most important functions is the display of surfaces in many different perspectives and configurations. These include fiducial, inflated, spherical, and flat maps. Generated maps can then be registered to an atlas and later used to make comparisons across different cases, such as normal and post-lesioned animals.

To quantify the distribution of synaptic terminal label resulting from our tracer injections, we will use Object Image software, which is based upon NIH Image, for generating an optical density measurements similar to that described by Henkel and colleagues (Henkel et al., 2003). Low magnification digital images of regions of terminal label will be acquired with an RT Slider camera (Diagnostic Instruments). These images will then be normalized to 255 grey levels and smoothed using a Gaussian filter. Using the line selection tool in Object Image, two series of equally spaced rectangular regions of interest, one encompassing the width and one encompassing the length of the terminal field, will be selected and a plot profile of the grey values will be generated and exported to a spreadsheet (Microsoft Excel). The length and width of the bands will be measured as the half-height of the peak in the profile for each sample. A series of high magnification images will be obtained in order to quantify the density of synaptic boutons within the terminal fields described above. These images will be converted to threshold values, and the "analyze particles" tool will be used to count the number of labeled boutons within each region of interest. The size and density measurements will be repeated for all terminal fields observed within each series of tissue.
E. Human Subjects

NA

F. Vertebrate Animals

Proposed use of animals

We will use 19 macaque monkeys (*Macaca mulatta*) over a five-year period. Five animals will be used for normal mapping experiments, 5 animals will be used for connections experiments in normal animals, and 9 animals will be used in 3 groups of behavior/lesion experiments. Given that we plan to examine the detailed topographic organization and connections of areas 5 and 2, as well as examine the behavioral consequences and cortical reorganization from lesions to area 5, the number of animals to be used is not excessive. Indeed, because some of the experiments can be combined in a single animal, we will maximize the use of each one, and thus use fewer animals. For example, in these studies, injections of three different tracers will be placed into 3 three different body parts of the same field. Further, the nine animals which will undergo lesions, will also be used to study the functional reorganization of cortical maps and connections. Our laboratory has extensive experience with these types of experiments on a number of different species, including Old World primates, and we can perform them with minimal distress to the animals. Most animals recover from the surgery very rapidly (2-6 hours postoperatively). All experiments in which the animals are allowed to recover will be done under sterile conditions at the California National Primate Research Center in Davis, CA.

Animals Justification

We have chosen macaque monkeys for two reasons. A). As Old World Cercopithecoid primates, macaque monkeys are relatively closely related to humans, and some of features of their cortex have recently been identified in humans using imaging techniques (Burton et al., 1993; Ledberg et al., 1995). Also, the glabrous hand with opposable thumbs is similar in both species. B). Several studies on cortical and thalamic organization and connections have already been done on this species in our laboratory. Thus, anesthetics levels, surgical preparation, transport time for anatomical tracers, locations of fields in cortex, and the angle of approach of the electrode for electrophysiological recording have already been determined.

Veterinary Care

The animals will be housed in approved facilities at the California National Primate Research Center (CNPRC) or building of the Neuroscience Center, and all recovery experiments will be carried out at the CNCRP. Throughout these experiments, the animals will be closely monitored by as well as an assembly of highly trained technical staff. Personal Info has access to the animals during all phases of the experiments. All experiments are approved by the University of California Animal Use and Care Administrative Advisory Committee (AUCAAC), and members of this committee have access to the animals during all phases of our experiments.

Alleviation of Pain and Distress

All sterile surgical procedures will be performed in approved suites, and all procedures will adhere to the NIH Guideline for the Care and Use of Laboratory Animals and AUCAAC. The animals will be anesthetized throughout all phases of the experiments, and for recovery experiments, closely monitored not only by the PI, but also by the Primate Center veterinarian and the trained staff at the Primate Center. Close postoperative monitoring will follow all recovery surgeries, and analgesics will be administered as needed during this time. Both pre-operative and postoperative administration of antibiotics will be given to prevent infection and related stress and discomfort.

Method of Euthanasia

At the end of all experiments, the animals will be given a lethal dose of pentobarbital (consistent with the recommendations of the American Veterinary Medical Association) and perfused with fixatives.
G. Literature Cited


CHECKLIST

TYPE OF APPLICATION (Check all that apply.)

☐ NEW application. (This application is being submitted to the PHS for the first time.)

☐ SBIR Phase I ☐ SBIR Phase II: SBIR Phase I Grant No. ☐ STTR Phase I ☐ STTR Phase II: STTR Phase I Grant No. ☐ SBIR Fast Track ☐ STTR Fast Track

☐ REVISION of application number: 1R01-NS035103

☐ COMPETING CONTINUATION of grant number: ________________________________

☐ INVENTIONS AND PATENTS

☐ (This application is to extend a funded grant beyond its current project period.)

☐ No. ☐ Previously reported

☐ Yes. If "Yes," ______

☐ SUPPLEMENT to grant number: ________________________________

☐ (This application is for additional funds to supplement a currently funded grant.)

☐ No. ☐ Previously reported

☐ CHANGE of principal investigator/program director.

☐ Name of former principal investigator/program director:

☐ FOREIGN application or significant foreign component.

1. PROGRAM INCOME (See Instructions.)

All applications must indicate whether program income is anticipated during the period(s) for which grant support is requested. If program income is anticipated, use the format below to reflect the amount and source(s).

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2. ASSURANCES/CERTIFICATIONS (See Instructions.)

The following assurances/certifications are made and verified by the signature of the Official Signing Applicant Organization on the Face Page of the application. Descriptions of individual assurances/certifications are provided in Section III. If unable to certify compliance, where applicable, provide an explanation and place it after this page.

- Debarment and Suspension
- Drug-Free Workplace (applicable to new Type I or revised Type I applications only)
- Lobbying
- Non-Delegation of Federal Debt
- Research Misconduct
- Civil Rights (Form HHS 441 or HHS 690)
- Handicapped Individuals (Form HHS 641 or HHS 690)
- Sex Discrimination (Form HHS 539-A or HHS 690)
- Age Discrimination (Form HHS 680 or HHS 690)
- Recombinant DNA and Human Gene Transfer Research
- Financial Conflict of Interest (except Phase I SBIR/STTR)
- STTR ONLY: Certification of Research Institution Participation

3. FACILITIES AND ADMINISTRATIVE COSTS (F&A/INDIRECT COSTS. See specific instructions.

☐ DHHS Agreement dated: 2/25/99

☐ No Facilities And Administrative Costs Requested.

☐ DHHS Agreement being negotiated with Regional Office.

☐ No DHHS Agreement, but rate established with Date

CALCULATION* (The entire grant application, including the Checklist, will be reproduced and provided to peer reviewers as confidential information.)

a. Initial budget period:
   
   Amount of base $ 202,640 x Rate applied 49.50 % = F&A costs $ 100,307

b. 02 year
   
   Amount of base $ 185,875 x Rate applied 50.50 % = F&A costs $ 93,867

c. 03 year
   
   Amount of base $ 185,875 x Rate applied 51.50 % = F&A costs $ 95,726

d. 04 year
   
   Amount of base $ 185,875 x Rate applied 52.50 % = F&A costs $ 97,584

e. 05 year
   
   Amount of base $ 185,875 x Rate applied 52.50 % = F&A costs $ 97,584

TOTAL F&A Costs $ 485,068

*Check appropriate box(es):

☐ Salary and wages base

☒ Modified total direct cost base

☐ Other base (Explain)

Explanation (Attach separate sheet, if necessary):

4. SMOKE-FREE WORKPLACE ☒ Yes ☐ No (The response to this question has no impact on the review or funding of this application.)