************************ NOTICE OF GRANT AWARD ************************

RESEARCH
Department of Health and Human Services
National Institutes of Health
NATIONAL INSTITUTE OF NEUROLOGICAL DISORDERS AND STROKE

Grant Number: 1 R01 NS051825-01 (Revised)
Principal Investigator: CHENEY, PAUL D PHD
Project Title: Electrical Stimulation of Cortical Motor Output

DIRECTOR, SPONSORED PROGS ADMIN
UNIV OF KANSAS MEDICAL CENTER
RESEARCH INSTITUTE, INC
3901 RAINBOW BLVD, Personal Info
KANSAS CITY, KS 66160
UNITED STATES
Award e-mailed to: Personal Info

Budget Period: 04/15/2005 - 03/31/2006
Project Period: 04/15/2005 - 03/31/2009

Dear Business Official:

The National Institutes of Health hereby revises this award (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF KANSAS MEDICAL CENTER RESEARCH INSTITUTE 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.

Sincerely yours,
Rita Sisco  
Grants Management Officer  
NATIONAL INSTITUTE OF NEUROLOGICAL DISORDERS AND STROKE

See additional information below

SECTION I - AWARD DATA - 1 R01 NS051825-01 (Revised)

AWARD CALCULATION (U.S. Dollars):
Federal Direct Costs $231,250
Federal F&A Costs $108,688
APPROVED BUDGET $339,938
TOTAL FEDERAL AWARD AMOUNT $339,938

AMOUNT OF THIS ACTION (FEDERAL SHARE) +$0

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project, is as follows:
02 $339,938
03 $339,938
04 $339,938

FISCAL INFORMATION:
CFDA 93.853
Number:
EIN: [redacted]
Document Number: RNS051825A


NIH ADMINISTRATIVE DATA:
PCC: CHEND CN / OC: 41.4A /Processed: SISCOR 050429 0342

SECTION II - PAYMENT/HOTLINE INFORMATION - 1 R01 NS051825-01 (Revised)

For Payment and HHS Office of Inspector General Hotline Information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III - TERMS AND CONDITIONS - 1 R01 NS051825-01 (Revised)

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.

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 - REVISED

This award is being revised to correct the Institutions mail address.

THE PREVIOUS TERMS AND CONDITIONS STATED BELOW REMAIN IN EFFECT.

Although the budget period start date for this award is 4/15/2005, this award includes funds for 12 months of support. Future year budget periods will cycle on 4/1. Allowable preaward costs may be charged to this award, in accordance with the conditions outlined in the NIH Grants Policy Statement (revised March 2001) and with institutional requirements for prior approval. The NIH Grants Policy Statement can be found on the internet at http://grants1.nih.gov/grants/policy/nihgps_2003/index.htm.

Documents (other than future year non-competing continuation applications) applicable to this grant should be faxed to (301)451-5635 or mailed to:

Grants Management Branch
National Institutes of Neurological Disorders and Stroke
6001 Executive Boulevard, Suite 3290, MSC 9537
Rockville, MD 20852 (Express Mail)
Bethesda, MD 20892-9537 (Regular Mail)

Future year non-competing continuation applications should be submitted to the new centralized mailing address for all NIH Institutes/Centers:

Division of Extramural Activities Support, OER
National Institutes of Health
6705 Rockledge Drive, Room 2207, MSC 7987
Bethesda, MD 20892-7987 (for regular or US Postal Service Express mail)
Bethesda, MD 20817 (for other courier/express mail delivery only)

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...
Daofen Chen, Program Official  
Phone: 301-496-1917  Email: dc342b@nih.gov  Fax: 301-402-1501

Aaron Kinchen, Grants Specialist  
Phone: 301-496-7386  Email: ak2840@nih.gov  Fax: 301-402-0219

SPREADSHEET

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| TOTAL COST | 339,938 | 339,938 | 339,938 | 339,938 |

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| F&A Cost Rate 1 | 47.00% | 47.00% | 47.00% | 47.00% |
| F&A Cost Base 1 | 231,250 | 231,250 | 231,250 | 231,250 |

END OF NGA

**************************** NOTICE OF GRANT AWARD ****************************
RESEARCH  
Department of Health and Human Services  
National Institutes of Health  
NATIONAL INSTITUTE OF NEUROLOGICAL DISORDERS AND STROKE  

Grant Number: 1 R01 NS051825-01  
Principal Investigator: CHENEY, PAUL D PHD  
Project Title: Electrical Stimulation of Cortical Motor Output
Budget Period: 04/15/2005 - 03/31/2006
Project Period: 04/15/2005 - 03/31/2009

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Rita Sisco
Grants Management Officer
NATIONAL INSTITUTE OF NEUROLOGICAL DISORDERS AND STROKE

See additional information below

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Federal Direct Costs $231,250
Federal F&A Costs $108,688
APPROVED BUDGET $339,938
TOTAL FEDERAL AWARD AMOUNT $339,938

Recommended future year total cost support, subject to the

http://avrmx7.cit.nih.gov/projindex.cfm?ApplId=6024754&requesttimeout=180
04/03/2009
availability of funds and satisfactory progress of the project, is as follows.
02 $339,938
03 $339,938
04 $339,938

FISCAL INFORMATION:
CFDA 93.853

EIN: EN
Document Number: RMS051825A


NIH ADMINISTRATIVE DATA:
PCC: CHEND CN / OC: 41.4A /Processed: SISCOR 050404 1136

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Daofen Chen, Program Official
Phone: 301-496-1917  Email: dc342b@nih.gov  Fax: 301-402-1501

Aaron Kinchen, Grants Specialist
Phone: 301-496-7386  Email: ak284o@nih.gov  Fax: 301-402-0219

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P.I.: CHENEY, PAUL D
INSTITUTION: UNIVERSITY OF KANSAS MEDICAL CENTER RESEARCH INSTITUTE

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..........................END OF NGA..........................
1. TITLE OF PROJECT
Electrical Stimulation of Cortical Motor Output

2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION
(if "Yes," state number and title)
No

3. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR

3a. NAME (Last, first, middle)
Cheney, Paul D.

3b. DEGREE(S)
Ph.D.

3c. POSITION TITLE
Professor

3d. Mailing address (Street, city, state, zip code)
Dept of Molecular and Integrative Physiology
University of Kansas Medical Center
3901 Rainbow Boulevard
Kansas City, KS 66160

3f. MAJOR SUBDIVISION
School of Medicine

5. VERTEBRATE ANIMALS
Yes

6. DATES OF PROPOSED PERIOD OF SUPPORT (month, day, year–MM/DD/YY)
From 04/01/05 Through 03/31/10

7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD
7a. Direct Costs ($)
250,000

8. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT
7b. Total Costs ($)
367,500
8a. Direct Costs ($)
1,250,000
8b. Total Costs ($)
1,837,500

13. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION

Name
Director, Sponsored Programs Administration

Address
Research Institute, Inc.
3901 Rainbow Boulevard
Kansas City, KS 66160

SIGNATURE OF PI/PD NAMED IN 3a.

6/28/04

DATE

Face Page

Form Page 1
Graziano et al. (2002a) recently demonstrated that applying repetitive intracranial microstimulation (RL-ICMS) to cortical motor areas for long durations (500 ms), matching the duration of normal movements, produces natural appearing arm movements ending with the hand positioned in different parts of extrapersonal space, depending on the cortical subregion stimulated. Three subregions were identified as producing movements with different characteristics. RL-ICMS of primary motor cortex (M1) evoked movements ending with the hand positioned in central space immediately in front of the monkey's chest and the formation of various postures of the digits appropriate for object manipulation. It was also reported that the pattern of EMG activity from stimulation was arm posture dependent and could switch from excitation to inhibition depending on initial posture. These findings were viewed as consistent with the hand reaching the same final position independent of the initial starting position. More recently, Graziano et al. (2004), reported that stimulus evoked output effects were also highly joint angle dependent. These results are remarkable and suggest a novel view of frontal lobe motor function. RL-ICMS was viewed as engaging functional neural substrates for natural, purposeful movements. In this application we will use stimulus triggered averaging of EMG and focus on M1 cortex to investigate some of the findings from RL-ICMS and to test possible alternative explanations for the findings observed. Four specific aims are proposed to rigorously test: 1) the extent to which EMG responses are limb posture and joint angle dependent, 2) the possibility that natural appearing responses observed with RL-ICMS can be explained by sustained, tonic coactivation of a particular set of muscles at each joint that simply achieve a final equilibrium position, and 3) the extent to which muscles activated with RL-ICMS can be explained based on detailed knowledge of M1 muscle maps and the pattern of spread of excitation through these muscle representations associated with ICMS.

PERFORMANCE SITE(S) (organization, city, state)

The University of Kansas Medical Center, Kansas City, Kansas
RESEARCH GRANT

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   Inclusion of Minorities (Required if Item 4 on the Face Page is marked “Yes”)
   Inclusion of Children (Required if Item 4 on the Face Page is marked “Yes”)
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Appendix (Five collated sets. No page numbering necessary for Appendix.)

Appendices NOT PERMITTED for Phase I SBIR/STTR unless specifically solicited □

Number of publications and manuscripts accepted for publication (not to exceed 10)

Other Items (list):

NONE
BUDGET JUSTIFICATION PAGE
MODULAR RESEARCH GRANT APPLICATION

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Total Direct Costs Requested for Entire Project Period: $1,250,000

Personnel
Paul Cheney, Ph.D., Principal Investigator, will be responsible for design and execution of all aspects of the project. He will work closely with and the graduate students in the daily conduct of data collection, data analysis, experimental design and the development of new procedures. He also leads all chamber and EMG implant surgeries and participates in many recording sessions. Dr. Cheney is also directly involved in writing all manuscripts.
Personnel continued

Consortium
NONE

Fee (SBIR/STTR Only)
NOT APPLICABLE
BIographical Sketch

Provide the following information for the key personnel in the order listed on Form Page 2. Photocopy this page or follow this format for each person.

NAME
Paul D. Cheney

POSITION TITLE
Professor and Chair
Department of Molecular & Integrative Physiology

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

<table>
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<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE (if applicable)</th>
<th>YEAR(s)</th>
<th>FIELD OF STUDY</th>
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<td>1969</td>
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<td>SUNY, Upstate Medical University, Syracuse, NY</td>
<td>Ph.D.</td>
<td>1975</td>
<td>Physiology</td>
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<tr>
<td>University of Washington School of Medicine</td>
<td>Postdoc</td>
<td>1974-1977</td>
<td>Neurophysiology</td>
</tr>
</tbody>
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A. Positions and Honors

Professional Experience
1977-1978 Research Assistant Professor, Department of Physiology and Biophysics, University of Washington School of Medicine
1978-1983 Assistant Professor, Department of Physiology, University of Kansas Medical Center, Kansas City, Kansas
1983-1988 Associate Professor, Department of Physiology, University of Kansas Medical Center
1988-present Professor, Department of Molecular and Integrative Physiology, University of Kansas Medical Center
1989-2002 Director, Smith Mental Retardation and Human Development Research Center, University of Kansas Medical Center and Co-director, Kansas Mental Retardation and Developmental Disabilities Research Center
2001-2002 Interim Chair, Department of Molecular and Integrative Physiology
2002-present Chair, Department of Molecular and Integrative Physiology

Honors and Other Professional Activities
Invited speaker at 18 national and international symposia.
Magna Cum Laude, State University of New York, College at Fredonia, 1969
Winner of the 1986-1987 Outstanding Educator Award from KUMC Medical Student Association.
Winner of the 1990-1991 Outstanding Instructor Award from KUMC Student Voice.
Member, NIH Study Section – Sensory Motor Integration (IFCN#5): June 1998 - present
Member, NIH-AARR#2 (AIDS and Related Research Study Section) – ad hoc reviewer 1999, 2000, 2001
Member, NIH-NCCR: Site visit review team for the University of Washington Regional Primate Research Center, Seattle, Washington, two separate five year reviews, Aug. 19-22, 1996; January 14-17, 2002
Member, Society for Neuroscience, Committee for the Development of Women’s Career’s in Neuroscience, 2001-present

B. Publications (selected from a total of 61)


C. Research support

Ongoing:

1. R01 NS 39023-03 (P. Cheney, PI) 9/15/99 - 8/31/04 (current year is no cost extension)
   NIH-NINDS
   Corticospinal control of forelimb movement.
   Purpose: The objective of this grant is to investigate basic issues related to the output organization of motor
   cortex in primates.
   Role: PI

2. R01 NDA12827-03 (P. Cheney, PI) 9/30/99 - 8/31/05
   NIDA
   Neuro-AIDS in opiate dependent rhesus macaques.
   Purpose: The objective of this grant is to the hypothesis that opiate dependence and withdrawal enhances
   the rate of progression and severity of neuro-AIDS.
   Role: PI

Completed:

1. R01 NS 38405-04 (P. Cheney, PI) 3/1/99 - 2/28/04
   NIH-NINDS
   Functional studies of brain infection with neurovirulent SIV in rhesus macaques.
   Purpose: The objective of this grant is to test the hypothesis that excitotoxicity is a contributing factor to
   neuronal injury in lentiviral disease.
   Role: PI
BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Photocopy this page or follow this format for each person.

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.

Laboratory:
Approximately 1200 sq ft of laboratory space consisting of two rooms in the will be fully dedicated to this project. One lab is fully instrumented for primate behavior and electrophysiological recording and electrical stimulation studies. A second lab is instrumented for behavioral training and will soon be fully equipped for electrophysiological recording. Both labs have sound attenuating chambers for monkey behavior.

Clinical:
N/A

Animal:
Animals are housed in a fully AAALAC accredited research facility located. Monkeys are housed in large cages and transported to the lab on the first floor in custom made primate chairs. Three veterinarians are available within the Laboratory Animal Resource facility to assist with care of the monkeys.

Computer:
Data collection in both labs is accomplished with two PC computers connected to CED hardware running custom software. Numerous additional PC computers support behavioral programs, data analysis, graphics, and document preparation.

Office:
Dr. Cheney occupies a 300 sq. ft. office in the Physiology Department.

Other:
Because Dr. Cheney is a member of the Kansas Mental Retardation and Developmental Disabilities funded by NICHD, this project will have access at a reduced cost to core services including graphics and illustration, histology services and statistical support.

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each. Behavioral equipment: 2 sound attenuating chambers, PC computers for data acquisition and behavioral control, custom primate chairs, custom torque motor based servo systems, pellet and applesauce feeders. Electrophysiological equipment: four Tektronix oscilloscopes, one CED Power 1401 data acquisition system and one 1401 plus data acquisition system in each lab. Both systems run custom Neural Averager software and one lab and a 28 channel TEAC in the second lab, each lab also has 26 Grass P511 amplifiers, sterilizer, Grass S88 stimulator with photic isolation units, Alpha Omega computer based spike discrimination system, 2 manual hydraulic microdrives, Grass audiomonitor.

The facilities of are also available to this project. This center includes a 3T Siemens head system and a 9.4T Varian animal system. We have used both for imaging the brains in our rhesus monkeys.
Research Plan

Abbreviations
ICMS: Intracranial microstimulation. Refers to any of a number of different methods in which electrical stimuli are delivered through a microelectrode suitable for recording from single units.
R-ICMS: Repetitive intracranial microstimulation. Stimulus frequency is generally 100-400 Hz, typically 330 Hz.
RL-ICMS: Long duration repetitive intracranial microstimulation (typically, 500 ms duration and 100-400 Hz)
RS-ICMS: Conventional short duration repetitive intracranial microstimulation (typically, 10 pulses at 330 Hz)
SITA: Stimulus triggered averaging of EMG activity. Typically 1-60 µA at a frequency of 20 Hz or less to avoid temporal summation with stimuli superimposed on background EMG activity associated with movements.

M1: Primary motor cortex

A. Specific aims

Graziano et al. (2002a) recently showed that applying intracranial microstimulation (ICMS) to frontal lobe motor areas, including primary motor cortex (M1), for durations similar to the durations of normal movements (500 ms, RL-ICMS), produced natural appearing arm movements ending with the hand positioned in different parts of extrapersonal space. One interpretation of RL-ICMS evoked "purposeful" movements, favored by Graziano et al. (2002a), is that RL-ICMS produces activation of underlying brain circuits, which mimic their normal activation, and yield the natural appearing movements observed (Graziano et al., 2002a,b). However, an alternative, simpler interpretation which is dismissed by Graziano et al. (2002b), is that the movements produced by RL-ICMS simply reflect an equilibrium process in which sustained, tonic contraction of agonist and antagonist muscles produce joint movement until the forces they produce come into balance as a result of movement along their respective length-tension relationships. In this case, the pattern of muscle activation is not the result of mimicking the normal activation of specific brain circuits, but rather reflects the orderly spread of excitation to multiple populations of corticospinal output neurons.

Another issue raised by the results of RL-ICMS experiments is the possibility that effects on muscle activity with ICMS methods are task dependent and may change dramatically with arm posture. This is based on a finding by Graziano et al. (2002a) that RL-ICMS effects on EMG activity can switch from excitation to inhibition depending on the posture of the arm relative to the final RL-ICMS evoked posture. In a more recent paper, Graziano et al. (2004) tested responses in triceps and biceps at different elbow angles from stimulation of M1 sites in the ketamine sedated monkey. They reported that output effects to these elbow muscles are highly joint angle dependent with triceps showing the strongest responses with the elbow flexed and biceps the strongest responses with the elbow extended.

The goal of the present proposal is to take advantage of the strengths of the approach we have applied extensively in the past, namely, stimulus triggered averaging of EMG activity from 24 chronically recorded forelimb muscles, to rigorously test hypotheses about underlying mechanisms involved in the responses associated with repetitive, long duration intracranial microstimulation (RL-ICMS) and the conclusion that responses from RL-ICMS and stimulus triggered averaging are highly dependent on task conditions. In this proposal, we will focus on primary motor cortex (M1). The findings from this work will be significant not only in terms of understanding fundamental properties of corticospinal output organization and the nature of motor output maps but also in terms of the mechanisms of output effects obtained with different forms of ICMS. The approach we will use is novel in its ability to delineate spatial maps for magnitude of output effects for large numbers of individual muscles in cortex. These maps will form the basis for interpreting the results obtained with RL-ICMS.

Specific Aim 1: to systematically map M1 cortical output to 24 muscles of the forelimb with SITA and RL-ICMS and to compare the profile of forelimb muscle activation (sign, strength and distribution) obtained with RL-ICMS with that from SITA of EMG activity at the same sites. Does RL-ICMS activate the same set of muscles as SITA at the same intensity? Is the specificity of muscle map organization lost when the parameters of RL-ICMS are applied? What are the movements, final arm postures and final hand positions
associated with RL-ICMS in the three subregions (distal muscle, proximal muscle, proximal-distal muscle combined) of the M1 forelimb representation described by Park et al. (2001)?

**Rationale and significance:** Our recent work demonstrates that M1 muscle maps based on StTA of EMG activity show consistent features including three subregions – a core area devoted to the representation of only distal muscles (wrist, digit, intrinsic hand), a horseshoe-like "surround" devoted only to proximal muscles (shoulder, elbow), and a zone from which combinations of distal and proximal muscles are co-activated as functional synergies. All recorded muscles were found to be represented in M1 but with broad overlap, particularly among muscles acting at the same joint. On the other hand, Graziano et al. (2002a) reported that RL-ICMS in M1 cortex evokes arm postures that bring the hand consistently to positions in front of the body. These responses often include associated movements of the hand and digits. It is difficult to fully reconcile the movements reported with RL-ICMS and our maps of muscle representation in M1. For example, Graziano et al. (2002a) did not report sites that produced only distal movements. All M1 sites produced an arm movement bringing the hand to a central location in front of the body, coupled with specific postures of the hand and digits. Neither were there sites that produced movements of the arm (proximal muscles) without associated movements of the hand and digits. We propose to examine this issue more carefully by systematically mapping M1 cortical output with both RL-ICMS and StTA. One question we hope to answer is whether basic features of map organization observed with low intensity StTA are preserved with RL-ICMS or does RL-ICMS produce broad and indiscriminate activation of muscle representations resulting in arm postures and hand positions that simply reflect the actions of the strongest muscles at each joint, for example, flexors. This aim will provide detailed data from systematic electrode penetrations of RL-ICMS evoked movements and associated patterns of EMG activity across all three forelimb subregions of M1.

**Approach and interpretation:** Our approach will be to first systematically map M1 cortex using StTA of EMG activity at 15 μA during a reach-to-grasp task as we have done in the past (Park et al., 2001). This will provide baseline data on the boundaries of M1 forelimb subregions (and maps of individual muscle representations) as well as boundaries with the trunk, leg and face representations. At all sites we will document the sign, strength and distribution of output effects to 24 muscles of the forelimb. From this data we will generate unfolded, two dimensional maps of each muscle's representation within M1. At each cortical site, we will also apply RL-ICMS with the monkey's arm in the starting location for the reach-to-grasp task. The arm is somewhat extended in this position but the muscles are relatively silent. RL-ICMS evoked final arm posture and hand position will be identified and starting arm positions will be tested that straddle and surround the final hand position. These experiments will provide systematic and detailed data on RL-ICMS evoked movements and EMG activity for identified subregions of the M1 forelimb representation. RL-ICMS evoked EMG activity will be analyzed in terms of the strength and distribution of effects and maps of individual muscles will be plotted as unfolded, two dimensional maps for comparison with StTA maps.

**Specific Aim:** to test the hypothesis that corticospinal output effects obtained with stimulus triggered averaging of EMG activity will not vary significantly with task conditions as long as EMG activity levels are similar. In contrast, output effects obtained with RL-ICMS will show changes in the sign (excitatory and inhibitory) and magnitude as a function of task conditions.

**Rationale and significance:** Are the output effects on muscle activity obtained with different forms of ICMS stable or do these effects show substantial changes as a function of task conditions, specifically, arm posture and joint angle? The results will have major implications for the interpretation of ICMS based mapping studies of cortical motor areas and for understanding the organization of corticospinal output.

**Approach and interpretation:** To test this hypothesis, we will compute StTAs of EMG activity with respect to 24 muscles of the forelimb from sites in each of the major subdivisions of the forelimb M1 representation (distal only, proximal only, proximal-distal cofacilitation) identified in our previous work (Park et al., 2002). We will generate StTA's during performance of an isometric wrist task and an isometric arm push-pull task.
For the wrist task, the monkey will produce isometric ramp-and-hold force trajectories alternately between flexion and extension. Data will be collected at five different wrist positions covering the range from flexion to extension. Particular attention will be focused on matching the levels of EMG activity, and hence, motoneuron excitability, for each of the joint positions tested. For the arm push-pull task, the monkey will grasp a knob with its hand and produce isometric ramp-and-hold force trajectories by alternately pushing (arm extension) and pulling (arm flexion) on the knob. The push-pull manipulandum will be positioned at different locations relative to the arm posture/hand location produced by RL-ICMS at each site. For both procedures, data will be saved to tape and averages will be compiled separately for the extension and flexion phases of movement. RL-ICMS data will also be collected for all hand positions in both the wrist and push-pull tasks. We predict that StTA effects will not show dramatic changes, for example, switching from excitation to inhibition or even substantial changes in magnitude, as a function of wrist joint angle or starting arm posture, although such task dependent modulation may be common with RL-ICMS evoked effects. We will interpret this result as consistent with the fact that StTA more directly reflects corticospinal output whereas RL-ICMS involves activation of neural circuits more broadly and the output effects from it may not reflect in any strict fashion, the local organization of corticospinal neurons at the site of stimulation.

Specific Aim 3: to test the hypothesis that RL-ICMS evoked "natural" movements result from the sustained tonic activity of a broad set of agonist and antagonist muscles whose actions move each joint to an equilibrium position based on the length-tension relationships of the activated muscles.

Rationale and significance: The results of RL-ICMS are subject to two different interpretations. One is that the movements produced appear to have normal qualities and appear purposeful because stimulation is activating a neural circuit that is normally used by the internal motor program to produce the same movement. An alternative interpretation is that RL-ICMS evokes a sustained and tonic contraction of agonist and antagonist muscles at multiple joints bringing the limb to a final posture that represents an equilibrium position between the forces generated by opposing muscles at each joint. We will focus on the following questions. Does RL-ICMS evoke sustained, tonic EMG activity in target muscles, consistent with an equilibrium position hypothesis, or does the EMG activity during RL-ICMS show time and position dependent EMG modulation similar to that associated with the monkey's own active movements and consistent with activation of a specific neural circuit for producing a natural, purposeful movement?

Approach and interpretation: To test this hypothesis, we will compare the pattern of muscle activation (magnitude and distribution across 24 muscles) as well as temporal profile of EMG activation for RL-ICMS evoked movements and for active movements with a similar trajectory initiated by the monkey. We will test selected sites within each of the subregions of the M1 forelimb representation. Are the same muscles activated and with similar amplitude and temporal characteristics for RL-ICMS evoked movements compared to the monkey's self initiated active movements? Substantially dissimilar patterns of EMG activation for RL-ICMS evoked and the monkey's own natural active movements will be interpreted as evidence that RL-ICMS does not activate a natural circuit for the movement produced. This will be reinforced by finding that RL-ICMS evoked muscle activity is highly stereotyped with sustained, relatively tonic activation lasting the duration of stimulation. This finding would support the view that the muscles activated by RL-ICMS produce joint movement toward an equilibrium position along their length-tension relationships in which opposing forces come into balance.

Specific Aim 4: to more fully and systematically document the relationships between stimulus parameters (intensity, frequency and train duration) and the sign, strength and distribution of output effects evoked with StTA, RS-ICMS and RL-ICMS. We will also determine the extent to which output effects with StTA and other forms of ICMS can be accounted for based on principles of physical stimulus spread in M1 cortex coupled with a detailed knowledge of individual muscle representations.

Rationale and significance: ICMS in various forms is widely used for mapping cortical motor output. There are two fundamentally different forms of ICMS: 1) StTA which delivers stimuli at a low rate (20 Hz or less)
which largely avoids temporal summation of synaptic potentials and has the advantage that both excitatory and inhibitory events can be detected, and 2) repetitive ICMS (200-400 Hz) which has the advantage that effects on EMG activity can be evoked without the need for averaging and movement responses can be documented. Repetitive ICMS can be short in duration (generally 10 pulses) or of long duration (e.g., 500 ms) more closely matching the duration of natural voluntary movements. Despite the wide use of these methods, there have been no systematic studies of how the strength and distribution of effects on muscle activity compares between different methods or how output effects change with stimulus intensity, frequency and train duration. This data is fundamental and important to the interpretation of results from motor output mapping using ICMS and for selecting optimal stimulation parameters. The results will also provide at test of whether changes in output (strength of effects and distribution to different muscles) with increasing stimulus intensity can be accounted for based on knowledge of the spatial distribution of individual muscle maps in the cortex and models of physical current spread.

**Approach and interpretation:** Three monkeys will be used for these experiments. So the results can be meaningfully interpreted in relation to the known somatotopic subdivisions of the forelimb M1 representation, we will first systematically map the spatial extent and magnitude of M1 cortical output with respect to 24 muscles of the forelimb using SITA of EMG activity. This will provide maps of the geometry of each muscle's representation in the cortex as well as contours of the strength of output within each representation. Selected sites in each subregion of the M1 forelimb representation will be selected for further study. We will use both the isolated wrist movement task and the reach-to-grasp task. The wrist task provides long periods of relatively stable EMG activity whereas the reach-to-grasp task provides the opportunity to test output to a broader set of muscles. For SITA, stimulus intensity will be varied over a large range and changes in the strength and distribution of output effects will be determined. These representational stimulus intensities will be selected for studies of stimulus frequency and train duration. A 3-dimensional model of the geometry of the precentral gyrus will be constructed from MRIs and layer V maps of individual muscles (based on 15 μA SITA) will be superimposed on this model. The spread of excitatory current through this hypothetical tissue volume will be modeled and used to interpret relationships between stimulus intensity and the magnitude of effects in individual muscles as well as changes in the distribution of effects to different muscles (muscle field) with increasing stimulus intensity. At each site, data will also be collected for RS-ICMS and RL-ICMS procedures. The data obtained from these experiments will provide a stronger foundation for the use of ICMS in cortical mapping. The modeling studies will also provide a test of the extent to which the magnitude and distribution of effects to different forelimb muscles is consistent with simple physical spread of current through the cortical tissue volume.

**B. Background and Significance**

Mapping cortical motor output with electrical stimulation has a long history dating back the second half of the 1800's (Cheney, 2002). Initial approaches were relatively crude but demonstrated that movements could be evoked in animals by stimulating specific parts of the cerebral cortex. These studies also revealed some basic features of somatotopic organization of motor cortex. More recent methodological advances have produced substantial new insight concerning the organization of motor function in the frontal lobe. One of these advances was the introduction by Asanuma and colleagues (Stoney et al., 1968) of intracranial microstimulation (ICMS). This method consists of applying electrical stimuli through a recording microelectrode to produce more focal activation of cortical output neurons. In the years since its introduction, ICMS has become widely used for mapping motor output and for answering other questions related to the nature of motor output both from the cortex and from other parts of the brain motor system (Cheney, 2002). ICMS typically consists of applying a short train of relatively low intensity (1-40 μA), high frequency stimuli (10 pulses at 330 Hz) to the cortex to evoke brief twitches of the peripheral musculature that can be used to generate spatial maps. We have designated this procedure as short train, repetitive microstimulation (RS-ICMS). This approach has the advantage that it can be applied effectively in
anesthetized animals to generate complete and detailed maps of motor output representation based on the movements evoked (Nudo et al., 1996; Stepniewska et al., 1993).

Another significant development in ICMS approaches to studying motor output function of the brain was the introduction of stimulus triggered averaging of EMG activity (StTA) by Cheney and Fetz (1985). StTA consists of applying microstimuli through a recording microelectrode but at a low frequency (20 Hz or less) to avoid temporal summation of effects and generally low intensity (40 µA or less). Because individual microstimuli are subthreshold for discharging motoneurons, StTA must be coupled with signal averaging and applied during active movements in the presence of background EMG activity.

A major advantage of StTA is that it produces output effects that closely match effects from single cells at the same site obtained with spike triggered averaging (see Preliminary Data section, Figure 4). This is true despite the fact that it is widely believed that the output effects from ICMS and StTA are mediated by transynaptic activation of corticospinal neurons. Nevertheless, data from StTA, can be interpreted as revealing the most elemental and direct units of cortical output. Accordingly, we predict that the effects obtained with StTA will be relatively invariant with task conditions, assuming EMG activity is similar under different conditions. At the same time, we acknowledge the fact that excitation of corticospinal output neurons with StTA appears to be largely transynaptic and this does present an opportunity for task dependent changes in efficacy at the cortical level. Also, it is certainly possible that changes in the size of muscle map representations observed with RS-ICMS (Donoghue et al., 1992) in relation to use, learning and injury might also be observed with StTA, although this has yet to be demonstrated.

In a recent paper, Graziano et al. (2002a) showed that applying intracranial microstimulation (ICMS) to precentral motor cortex for relatively long durations (500 ms, RL-ICMS) produced natural appearing arm movements ending with the hand positioned in different parts of extrapersonal space depending on the area of frontal cortex stimulated. Stimulation of sites in medial premotor cortex produced movements that seemed to have a defensive purpose. Sites corresponding to lateral premotor cortex produced hand-to-mouth movements coupled with hand grasp, resembling movements associated with feeding, whereas stimulation at sites corresponding to primary motor cortex (M1) produced arm movements bringing the hand to final positions located in central space immediately in front of the monkey’s chest. These responses were often accompanied by movements of the digits resulting in a variety of hand postures resembling precision grip, power grip and opening of the hand with the digits splayed. The movements were described as similar to natural movements involved in visually guided manipulation of objects. The durations and velocity profiles of RL-ICMS evoked movements were also similar to natural movements.

Graziano et al. (2002a) further reported that the EMG pattern in a particular muscle from stimulation at a cortical site could switch from excitation to inhibition depending on the initial posture of the arm. Switching from excitation to suppression was interpreted as consistent with the finding that for stimulation of a given cortical site, the hand achieves a specific final position independent of the starting location. Achieving this final position might require extension of the arm if the starting position was a more flexed position than the final target location, or flexion if the arm’s starting position was more extended than the final position.

In a more recent paper, Graziano et al. (2004) tested responses in triceps and biceps at different elbow angles from stimulation of M1 sites in the ketamine sedated monkey. They reported that output effects to these elbow muscles are highly joint angle dependent with triceps showing the strongest responses with the elbow flexed and biceps the strongest responses with the elbow extended. A similar dependence on joint angle was observed with both RL-ICMS and StTA. Although this result is completely consistent with changes in motoneuron excitability associated with changing levels of muscle spindle afferent input, the authors instead prefer to interpret their results as supporting a map of “desired” arm postures in motor cortex, providing general support for their hypothesis that M1 neurons provide “higher order signals instructing the limb to move to a certain posture regardless of the initial posture”. These results also potentially raise general questions about the reproducibility of ICMS based mapping studies. For example,
the authors state that "each cortical site did not appear to have a fixed mapping to biceps or triceps". This work has major potential implications, not only for understanding the organization of M1 corticospinal output, but also for the application of ICMS methods to brain mapping. We feel it is essential that these findings be replicated and alternative interpretations tested.

Although Graziano et al. (2002a) frequently used relatively high stimulus intensities (e.g., 150 μA, although less in M1) to elicit movements, the results are, nevertheless, quite remarkable and present a novel view of frontal lobe motor function. However, the RL-ICMS results raise a number of issues and are subject to multiple interpretations with vastly different implications and significance. First, the observation of posture dependent switching from excitation to inhibition reported raises the question of whether this switching also occurs with StTA and short duration ICMS (RS-ICMS). Joint angle dependent modulation of output effects is a potentially important finding but only if it can be shown to occur in the absence of changes in motoneuron excitability due to changing proprioceptive input. These issues will be directly addressed by specific aim 2. Second, the observation of normal appearing, purposeful movements with RL-ICMS might be interpreted as evidence for the presence of underlying specialized brain circuits designed to bring the limb and hand to specific postures (Graziano et al., 2002a); however, it seems that a simpler interpretation has not been ruled out. An alternate interpretation would be that the movements obtained with RL-ICMS simply reflect an equilibrium process in which sustained, tonic contraction of agonist and antagonist muscles produce joint movement until their forces come into equilibrium. The muscles activated are not the targets of a specialized brain circuit for specific types of movements but rather reflect the orderly spread of excitation to multiple populations of corticospinal output neurons. Knowledge of the cortical output maps for individual muscles and the extent of stimulus spread might provide insight as to the movements produced with RL-ICMS. This issue will be addressed by specific aim 2. Third, the movements produced with RL-ICMS from sites in M1 are not distributed over the entire workspace but are focused on locations immediately in front of the monkey and often involve associated movements of the digits and hand. What muscles are activated with RL-ICMS and does RL-ICMS tend to produce predominant activation of certain muscles thus explaining the movements evoked? Our recent work identified three subregions of the M1 representation—a core area devoted to the representation of only distal muscles, a horseshoe-like "surround" devoted to only proximal muscles, and a zone separating the distal and proximal regions that from which combinations distal and proximal muscles can excited. All recorded muscles were found to be represented in M1 but with broad overlap. Does RL-ICMS at known locations within these representations produce predictable responses based on knowledge of the individual muscle maps or is any relationship to basic muscle map topograph lost?

C. Preliminary Data

This application is focused on the mechanisms and interpretation of responses obtained from primary motor cortex with RL-ICMS. This section contains a summary of the results obtained with RL-ICMS, a presentation of different methods of intracranial microstimulation and their interpretation, and a summary of recent results obtained in the PI's laboratory in which stimulus triggered averaging of EMG activity was used to map the spatial distributions of individual muscles in M1.

Forelimb movements and postures produced with RL-ICMS
Graziano et al. (2002a) recently showed that applying repetitive intracranial microstimulation (ICMS) to precentral motor cortex for relatively long durations (500 ms) produces natural appearing arm movements ending with the hand positioned in different parts of extrapersonal space depending on the cortical subregion stimulated (Figure 1). One subregion (roughly the dorsal part of F4) evoked movements that seem to have a defensive character, another subregion (probably a part of F5) evoked responses that emphasized grip postures of the hand and movements of the hand to the mouth as would occur during feeding behavior and a third subregion, corresponding to primary motor cortex (M1), evoked movements resulting in final hand positions in central space immediately in front of the monkey's chest. The responses
from M1 were often accompanied by movements of the digits resulting in a variety of hand postures including precision grip, power grip and opening the hand with the digits splayed. These movements resembled natural movements associated with visually guided manipulation of objects. Graziano and colleagues argue that these stimulus evoked movements “mimic normal ones”.

Figure 1A. Specialized subregions of RL-ICMS evoked arm postures based on data from one monkey. Circles show hand-to-mouth sites; these always involved a grip posture of the hand in addition to a movement of the arm that brought the hand to the mouth. Triangles show sites where RL-ICMS evoked both a hand and an arm posture; these sites often involved the hand moving into central space and the fingers shaping into a specific configuration. Squares show sites where bimodal, visual-tactile responses were found and stimulation evoked defensive movements. (From Graziano et al., 2002a)

Figure 1B. Summary map of RL-ICMS evoked movements. The region labeled “polysensory, defensive” (green) may correspond functionally to the dorsal part of area F4. The region labeled “hand-to-mouth, grasp” (red) may correspond to part of area F5. The region labeled “complex manipulation, emphasizes central space” (blue) may correspond to the forelimb representation of primary motor cortex (M1). (From Graziano et al. 2002b)

Arm posture and joint angle dependence of EMG responses to long duration ICMS
Graziano et al. (2002a) reported that the pattern of the EMG activity from stimulation at a particular cortical site could switch from excitation to inhibition depending on the initial posture of the arm. An example of their findings is reproduced in Figure 2. In this case, RL-ICMS with the elbow fully extended produced flexion to a final posture; with the elbow fully flexed, RL-ICMS produced extension to same final posture. RL-ICMS was then delivered to this cortical site while recording EMG activity from the biceps and triceps muscles. The arm was fixed in either an extended or flexed position. Note that with elbow in extension, the biceps is activated and triceps is weakly suppressed. This pattern reverses when RL-ICMS is delivered with the elbow flexed. This result was interpreted as consistent with the principle that cortical sites are mapping in terms of a final position in space, independent of the initial starting position. We will determine how commonly this type of switching occurs for sites in M1 and if it is dependent on the use of long duration stimulation and higher intensities.

Recently, Graziano et al. (2004) reported that the EMG responses in biceps and triceps were joint angle dependent. A typical result from their paper is shown in Figure 3. These results were obtained in ketamine tranquilized monkeys. There is no question that the magnitude of output effects obtained with both RL-ICMS and StTA were highly dependent on joint angle under the conditions of this experiment. Biceps facilitation (thick line) was strongest in the most extended position of the elbow, whereas triceps facilitation (thin line) was strongest in the most flexed position of the elbow. However, this result would be expected from changes in motoneuron excitability brought about by joint dependent changes in muscle spindle input.
to motoneurons. Specific aim 2 of the current proposal will test the joint angle dependence of output effects in the absence of anesthesia and under conditions in which motoneuron excitability at different joint angles can be matched.

Elbow Extended

Elbow Flexed

**Figure 2.** EMG activity from muscles of the upper arm during RL-ICMS (500 ms @ 100 μA) of one site in primary motor cortex with the arm in different initial postures (extended or flexed). Note that the responses obtained are strikingly different depending on initial posture. (From Graziano et al., 2002a)

**Figure 3.** Top: locations of 6 example sites in primary motor cortex; the enlarged drawing of the central sulcus is based on data from monkey 1. Data in A & B came from the site indicated by the circle and the arrow. The monkey was tranquilized with ketamine during testing. A: EMG activity of the biceps (thick line hardly visible, close to the baseline) and triceps (thin line in each histogram) muscles evoked by 200-Hz stimulation at 30 μA when the elbow was fixed at 4 different angles. Thick black line under each record indicates duration of stimulation train. Each record is a mean of 15 trials. Evoked activity in both muscles was significantly affected by elbow angle. B: EMG activity evoked by STTA at 15-Hz. Vertical line is the time of the stimulus. Time from 0.2 ms before to 1.5 ms was contaminated by artifact and has been removed from the records. Each STTA record is a mean of 2,000-4,500 stimuli. Evoked activity in both muscles was significantly affected by elbow angle in a manner similar to effects from RL-ICMS. (From Graziano et al., 2004)
Intracortical microstimulation (ICMS) methods

ICMS can take several different forms and can be coupled with a variety of output measures including evoked movements, evoked EMG responses and averaged EMG responses (Cheney, 2002). Standard repetitive ICMS (RS-ICMS) generally consists of 10 cathodal or biphasic pulses at 330 Hz (0.2 ms duration each phase). Using RS-ICMS to evoke EMG activity has the advantage that individual muscle responses can be resolved if EMGs are recorded. Stimulation can also be applied during movement to reveal inhibitory effects. EMG responses from stimulation can be averaged over several trials to reduce variability and provide clear records for quantitation and latency measurements.

Despite being the method of choice for many brain mapping experiments, RS-ICMS is not without weaknesses. First, it suffers from the same problems that exist with any electrical stimulation technique. Namely, stimulation activates a variety of neuronal elements including not only cell bodies but also axons of passage and afferent terminals. The effects of ICMS can potentially produce excitation at sites distant to the stimulation site by physiological spread of the stimulus resulting from temporal facilitation within the cortical circuitry. This is especially true for high frequency, repetitive ICMS. Jankowska et al. (1975) demonstrated this by applying ICMS to the site of a pyramidal tract neuron in the hindlimb area of motor cortex while monitoring the descending volley from a fascicle of the lateral corticospinal tract. Each successive stimulus of a train consisting of three stimuli evoked a descending volley that was larger in amplitude and greater in duration than the preceding stimulus. The most probable explanation of the increasing descending volley with R-ICMS is that corticospinal neurons are excited transsynaptically. Although direct activation of corticospinal neurons with ICMS certainly occurs (Stoney et al., 1968), the effects evoked with repetitive stimulation appear to be predominately from transynaptic excitation.

Spike and stimulus triggered averaging of EMG activity

Spike triggered averaging of rectified EMG activity was introduced by Fetz and Cheney (1980) and provides a method of identifying the target muscles of single corticospinal cells and the cells of other descending systems. This method has made it possible to investigate in awake animals, not only relations between a cell's discharge and movement, but also the synaptic organization between single cells and motoneurons of agonist and antagonist muscles (Cheney, 2002).

Spike triggered averaging of EMG activity is based on the rationale that spike discharges of neurons with a direct excitatory synaptic linkage to motoneurons will produce individual EPSPs at a fixed latency following discharge of the neuron. The magnitude of the EPSP will be too small to reliably discharge the motoneuron with each occurrence. Nevertheless, the EPSPs will depolarize the membrane, bringing the motoneuron closer to firing threshold and will transiently increase motoneuron firing probability. Since the neuromuscular junction is normally an obligatory synapse, a one-to-one relationship exists between motoneuron and muscle fiber action potentials. Therefore, motor unit spike trains directly reflect the firing of spinal motoneurons. EMG activity is the sum of the spike trains of a population of motor units within a muscle and provides a measure that can be used to detect changes in firing probability associated with the occurrence of neuronal cell spikes. Transient increases in average EMG activity associated with the spikes of neurons are referred to as postspike facilitation (PSpF) effects. PSpF is interpreted as evidence of an underlying synaptic linkage between the trigger neuron and motoneurons. Postspike suppression (PSpS) can also be detected and is often present in antagonists of muscles showing PSpF from the same cell. PSpS has a longer latency consistent with a minimum disynaptic linkage. Spike triggered averaging of EMG activity has now been used extensively to investigate synaptic relationships between cortical and red nucleus neurons and forelimb motoneurons (Cheney et al., 1991; Cheney, 2002; Fetz and Cheney, 1980; Fourment et al., 1995; Lemon et al., 1986; Sinkjaer et al., 1995; Poljakov and Schiebler, 1998).

In a manner analogous to spike triggered averaging of EMG activity, averages can also be referenced to stimuli applied to the sites of recorded cells. This method is referred to as stimulus triggered averaging (StTA) of EMG activity (Cheney and Fetz, 1985). In this procedure, averages are computed using the same parameters that were previously used for spike triggered averaging except that the computer is triggered
from microstimuli delivered at a low rate during movements of interest. The low rate of stimulation (5-20 Hz) avoids spread of excitation by temporal summation. Stimulation must be applied during active movements so EMG activity is present, and coupled with averaging because the individual stimuli are generally subthreshold for discharging motoneurons.

Stimulus triggered averages can be readily quantified. Onset latencies reflect conduction time and synaptic transmission in the anatomical pathway from the site of stimulation to the muscle. Latency data from StTA, therefore, is much more meaningful for interpretation purposes than onset latencies obtained with repetitive ICMS. Both excitatory and inhibitory events can be detected. It is important to remember that the effects obtained with StTA reflect the summation of all the stimulated elements including cell bodies activated directly by the stimulus, axon collaterals and afferent terminals to corticospinal neurons.

Cheney and Fetz (1985) reported that poststimulus facilitation (PSIF) in wrist and digit muscles has an onset latency that is about 1.3 ms longer than postspike facilitation in the same muscle from the same site. This is a consistent finding that has been replicated many times. The most likely explanation of this latency difference is that it reflects the fact that activation of corticospinal neurons with StTA is predominately transsynaptic rather than direct. Although this remains an issue that needs further investigation, it is always important to recognize the likely indirect nature of activation of corticospinal output with ICMS because this is very important in the interpretation of results. Nevertheless, even though activation may be largely indirect, StTA clearly produces a profile of effects in muscles that closely matches that from single cells at the same site (Cheney and Fetz, 1985). It is also clear that RS-ICMS at low intensity can produce a profile matching that of SpTA and StTA (Figure 4). One of the goals of this project will be to investigate the muscle output profile obtained with StTA and repetitive ICMS more systematically and determine, how the profile changes as higher intensities and longer durations of stimulation typical of RL-ICMS are applied.

**Comparison of results from spike triggered averaging, stimulus triggered averaging and RS-ICMS**

In the current proposal, it is postulated that output effects obtained with low intensity StTA reflects the activation of a highly localized cluster of corticospinal neurons. What is the evidence to support this

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**Figure 4.** Output effects on wrist and digit extensor muscles in spike triggered averages (SpTA) from cell in M1 cortex and from stimulus triggered averaging (single pulse ICMS) and RS-ICMS at the same site. Note that the profile of effects (muscles facilitated and relative strength of facilitation) obtained with both StTA and RS-ICMS.
closely match the profile obtained from a single cortical neuron with SpTA. SpTA and SITA data were collected during performance of a step tracking wrist task; RS-ICMS (right column) data were collected with the monkey sitting at rest, although similar results were obtained with RS-ICMS applied during the wrist movement task.

Conclusion? One of the most important pieces of evidence comes from comparing effects from spike triggered averaging, which reveals the output effects of single corticospinal cells, with the effects from SITA computed at the same cortical site. A typical example is illustrated in Figure 4. The basic result is that the pattern of poststimulus effects across different muscles at a particular cortical site closely matches the pattern of postspike facilitation (Cheney and Fetz, 1985). In this example, PSpF was strongest in ED4,5 and EDC but was also clear in ECU based on an average of 14,000 spike events. A SITA computed from 500 microstimuli at 5 μA applied to the same cortical site showed PSTF in the same muscles. Moreover, the rank order of PSTF by magnitude matched the rank order of PSpF. Although appearing comparable, the absolute magnitude of PSTF is actually much greater than PSpF because it was obtained with only 500 stimuli compared to 14,000 spikes for PSpF. Taking into consideration the fact that signal-to-noise ratio increases as the square root of the number of trigger events, the magnitude of PSTF in this case is actually about five times greater than PSpF. Whereas PSpF reflects the output organization of a single cell, PSTF reflects the output effects of the population of cells and other neuronal elements that are excited by the stimulus. The fact that PSTF involves many CM cells but has the same basic profile across synergist muscles as PSpF from a single CM cell at the same site suggests that neighboring cells activated by the stimulus have similar patterns of synaptic connections with motoneurons. The similarity in target muscle fields of neighboring CM cells has been confirmed by computing spike-triggered averages from adjacent cells recorded simultaneously through the same microelectrode (Cheney and Fetz, 1985). Based on these results, it seems reasonable to conclude that neighboring corticospinal cells in lamina V of motor cortex share a similar set of target muscles (the cell’s muscle field) and that output effects obtained with SITA reflect the local organization of corticospinal output neurons.

How do results from RS-ICMS compare with those from spike and stimulus triggered averaging? In Figure 4, averages from 40 trains of RS-ICMS (10 pulses @ 330 Hz and 10 μA) yielded the same profile of muscle effects as observed in spike and stimulus triggered averages. Despite the fact that RS-ICMS involves significant temporal summation and indirect excitation of corticospinal cells, in this case, facilitation remained confined to the same muscles. This suggests that in addition to the corticospinal cells directly activated by the stimulus, all stimulated neuronal elements related to the cell cluster may be part of a common functional unit linking the three activated muscles. If this were not true, then spread of excitation from repetitive stimulation would have certainly resulted in effects appearing in other muscles.

Finally, the nature of the RS-ICMS evoked movement was obtained for the site illustrated in Figure 4. The movement evoked was extension of digit four. Such a restricted movement response would have been difficult to predict from the results of spike triggered averaging, stimulus triggered averaging or RS-ICMS, although ED 4,5 did show the strongest facilitation. This example illustrates clearly the impossibility of drawing strong conclusions about the muscles activated in association with movements evoked by stimulation. To conclude, mapping based on EMG recording and averaging is very effective for revealing the distribution of output from the site of stimulation to individual muscles in a form that is reproducible and quantifiable. In the work proposed, we will build upon the work illustrated in Figure 4 and will add long duration ICMS to the protocol. The data obtained will not only be relevant to questions about the mechanism of RL-ICMS evoked movements, but will also provide systematic data for comparing the sign, distribution and strength of RS-ICMS and RL-ICMS output effects with those from stimulus triggered averaging.

Muscle maps of M1 cortex based on results with SITA of EMG activity

Figure 5 shows 2-D maps of the forelimb representation of M1 in two macaques. This data was obtained from systematic electrode penetrations at 1.0 mm spacing throughout the arm representation of the precentral gyrus to map the representation of each of these 24 muscles. Within each electrode track,
stimulation was applied every 0.5 mm. M1 sites yielding effects in only proximal (shoulder and elbow) muscles are represented by the red area, only distal (wrist, digit and intrinsic hand) muscles by the blue area and sites yielding effects in combinations of proximal and distal muscles are represented by the purple area. The green area is hindlimb and trunk and the blue area is face. In these maps, the precentral gyrus has been unfolded. The maps of distal and proximal muscle representation are consistent with results from retrograde labeling of corticospinal neurons following injections of tracers in the upper cervical (proximal muscles) and lower cervical (distal muscles) spinal cord (He et al., 1993). The central core of distal muscle representation surrounded by a zone of proximal muscle representation is a consistent feature of the intra-areal forelimb map in the monkey (Park et al., 2001). In addition, the maps in Figure 5 reveal for the first time a zone in which SITA produced effects in combinations of both proximal and distal muscles. The dimensions of this zone are not compatible with simple current spread from pure distal and pure proximal representations. The existence of a specific zone with sites representing proximal and distal muscles in different combinations is consistent our results from spike triggered averaging showing that about half of corticospinal cells involved in reach-to-grasp tasks facilitate at least one proximal and one distal muscle (McKiernan et al., 1998).
substrates for producing basic synergies underlying coordinated, multi-joint movements, for example, extending and withdrawing the limb.

Systematic mapping of M1 cortex with stimulus triggered averaging of EMG activity from 24 muscles of the forelimb yields data that can be used to generate complete maps of individual muscle representations. We will use this approach extensively for the specific aims of the current proposal.

EMGs from 24 muscles of the forelimb recorded simultaneously

Methods for recording EMG activity from relatively large numbers of forelimb muscles simultaneously have been detailed by Park et al (2000). Figure 7 shows EMG records from 24 simultaneously recorded forelimb muscles during a reach-to-grasp task. These are the same 24 forelimb muscles that will be recorded for the aims of the current proposal. Note that this task produces broad, overlapping activity in most of the 24 muscles, although EMGs for individual muscles show a fine structure that is often unique. The existence of
broad coactivation of all muscles makes this an ideal task for identifying the target muscles of individual cells with spike triggered averaging or small local clusters of cells with stimulus triggered averaging.

Figure 7. EMG activity from 24 simultaneously recorded forelimb muscles associated with performance of the reach-to-grasp task. The monkey begins from a home plate at waist height, reaches for a food pellet delivered to a well, takes the food pellet to its mouth and then returns to home plate. Entry into the food well is detected by infrared beams. Contact with home plate is detected by a switch closure (lower records). Single trial records show changes in rectified and filtered EMG activity for all 24 forelimb muscles during performance of the task. Shoulder, elbow, wrist, digit and intrinsic hand muscles were recorded. Although the records for APB and FDI look very similar, visual examination of the expanded records confirmed the lack of temporal synchronization in peaks (from McKiernan et al., 1998). Shoulder muscles: pectoralis major (PEC), anterior deltoid (ADE), posterior deltoid (PDE), teres major (TMAJ) and latissimus dorsi (LAT); elbow muscles: biceps short head (BIS), biceps long head (BIL), brachialis (BRA), brachioradialis (BR), triceps long head (TLON), triceps lateral head (TLAT) and dorsi-epitrochlearis (DE); wrist muscles: extensor carpi radialis (ECR), extensor carpi ulnaris (ECU), flexor carpi radialis (FCR), flexor carpi ulnaris (FCU) and palmaris longus (PL); digit muscles: extensor digitorum communis (EDC), extensor digitorum 2,3 (ED23), extensor digitorum 4,5 (ED45), flexor digitorum superficialis (FDS) and flexor digitorum profundus (FDP); and Intrinsic hand muscles: abductor pollicis brevis (APB) and first dorsal interosseus (FDI).

D. Research Design and Methods

The methods are organized around each of the specific aims. General methods that apply to all specific aims are presented at the end of this section.

Protocol for specific aim 1

Rationale and experimental design
The goal of this aim is to systematically map forelimb M1 cortex with respect to 1) SITA evoked EMG responses in 24 muscles of the forelimb, 2) RL-ICMS evoked EMG responses at the same cortical sites as SITA, and 3) movements evoked with RL-ICMS at the same cortical sites. A major question we hope to answer is the extent to which consistent features of map topography observed with SITA are retained with RL-ICMS and whether movements evoked with RL-ICMS are consistent with distinct M1 representations for distal muscles only, proximal muscles only and combined proximal-distal muscles.

Monkeys will be trained on the same reach-to-grasp task (Fig. 7) we have used extensively in previous work. This task has the advantage of producing broad coactivation of proximal and distal forearm muscles in a variety of synergies. All 24 recorded muscles are activated during some phase of this task. The presence of EMG activity is a requirement for successful SITA.

Three monkeys will be needed for this specific aim. Two monkeys might be sufficient but recent experience in which one of our monkeys died has confirmed the need for a backup. We will also use these monkeys for the data collection and analysis in specific aims 2 and 3. The reach-to-grasp task will provide a point of reference with our recent mapping work, which is all based on this task.

Data collection and protocol
1. Electrode penetrations will be made in M1 cortex following an orderly grid of 0.5 mm spacing. Within each electrode penetration, stimulation will be performed at 0.5 mm intervals. Sites most likely corresponding to layer V will be identified based on multiple criteria including depth, distance from white matter, reconstruction based on MRI, magnitude of effects relative to nearby stimulation sites (Park et al., 2002). SITAs at 15 μA will be collected on-line while the monkey performs the reach-to-grasp task. Averages will be based on at least 1,000 stimuli. In addition, all data will be recorded on a 32 channel TEAC instrumentation tape recorder for later off-line analysis as needed. This data will provide a map of the organization of corticospinal output and also a point of reference to our previous work, which was all based on this approach.

2. After collecting SITAs at 15 μA at a cortical site, we will apply RL-ICMS and quickly assess stimulus intensity to identify the minimum intensity needed to produce consistent movements to a reproducible final hand position and arm posture. RL-ICMS data will then be obtained, first with the hand in a standard starting position, namely home plate of the reach-to-grasp task. This device occupies a neutral position at waist level and about 20 cm immediately in front of the monkey. Previous work has demonstrated that this is a true resting position and involves little or no EMG activity in arm or hand muscles (Fig. 7). RL-ICMS will also be applied with the hand at other starting positions surrounding the preferred RL-ICMS final target position (Figure 8, blue dots and orange dots). A minimum of 5 trials will be collected and the EMG responses averaged. Stimulus artifacts (if any) will be removed from the EMG records; the signals will then be rectified and averaged. RL-ICMS evoked movements will be recorded using two digital video cameras – one positioned above the monkey and one to the monkeys side. Movement trajectories and final hand positions and arm postures will also be noted on line and confirmed from video tape playback. Monkeys will be enticed to the desired starting positions with food treats (e.g., raisins).

3. SITAs will be repeated using the RL-ICMS stimulus intensity applied in #2.

4. Using the SITA data obtained at 15 μA and the RL-ICMS intensity, unfolded, 2-D maps of individual muscle representations color coded for the strength of effects at different sites within the map will be constructed. Boundaries for distal, proximal and proximal-distal combined muscle subregions will be identified. 2-D maps will also be constructed for the RL-ICMS data. Individual muscle maps from SITA and RL-ICMS will be compared in terms of area, location, presence of non-contiguous representations and center of gravity (Park et al., 2002). RL-ICMS evoked movements, final hand positions and arm postures will be superimposed on the maps.
**Interpretation**

These experiments will provide systematic and detailed data on RL-ICMS evoked movements and EMG activity for known subregions of the M1 forelimb representation. Based on existing data it is difficult to predict the outcome of this experiment. The fact that Graziano et al. (2002a) did not report a region producing only distal or only proximal muscle responses suggests that with RL-ICMS the detailed and reproducible topography observed with STTA will be lost. However, it is possible they may have missed this part of the representation. We do expect to find distinct distal only, proximal only and combined proximal-distal representations with STTA at the stimulus intensity used for RL-ICMS. In any case, we will be able to document differences in map organization with these different forms of ICMS and the movements evoked with RL-ICMS from different subregions of the forelimb representation.

**Protocol for specific aim #2:**

The goal of this aim is to test the hypothesis that corticospinal output based on poststimulus effects in STTAs of EMG activity will not vary substantially with task conditions as long as EMG activity levels are similar, whereas effects obtained with RL-ICMS will show changes in the sign (excitatory and inhibitory) of output effects and/or magnitude of effects.

**Rationale and experimental design**

Graziano et al. (2002a) reported that EMG activation with RL-ICMS switched from excitation to inhibition depending on the position of the arm in relation to the final hand position obtained with stimulation. We postulate that such switching is a characteristic of the parameters of stimulation and will not occur with STTA, which more directly reflects the activation corticospinal output neurons and their spatial arrangement in the cortex (Cheney and Fetz, 1985). Most recently, Graziano et al. (2004) reported that the magnitude of output with RL-ICMS and STTA was strongly dependent on joint angle — stronger effects the more the muscle was stretched. However, interpretation of these observations is compromised by the fact that the monkey was tranquilized and the excitability of motoneurons is likely to have varied consistently as a function of joint angle. Again we postulate that this dependence on joint angle will not occur if the experiment is designed so as to maintain a constant level of motoneuron excitability at different joint angles.

To investigate this issue, we will use the three monkeys from specific aim 1, in which M1 cortex will have already been systematically mapped with respect to 24 forelimb muscles using STTA at 15 μA during the reach-to-grasp task. Using this map, selected sites in each of the three forelimb subregions will be identified to address the questions posed in specific aim 2. Monkeys will be trained on two additional tasks: 1) an isometric arm push-pull task (Figure 8) in which the monkey will grasp a knob with its hand and produce isometric ramp-and-hold force trajectories by alternately pushing (arm extension) and pulling (arm flexion) on the knob, and 2) an isometric wrist task (Figure 9) in which the monkey will generate isometric ramp-and-hold force trajectories alternately between flexion and extension with the goal of matching the level of EMG activity at each joint position. The arm push-pull task emphasizes proximal muscles whereas the wrist task uses exclusively distal muscles. Sites within each of the three subregions of the M1 forelimb representation will be tested with each task, but with emphasis on subregions containing distal representations for the wrist task and subregions containing proximal representations for the arm push-pull task.

**Specific aim 2 - experiment 1: isometric arm push-pull task (Figure 8) - switching of muscle output effects between excitation and inhibition for starting hand positions on opposite sides of the RL-ICMS evoked final hand position**

**Data collection and protocol:**

Data will be collected from at least 20 sites in each of the major forelimb subdivisions of M1 (distal only, proximal only and proximal-distal cofacilitation) as described by Park et al. (2001) and illustrated in Figure 5. The data to be collected at each site and the order of collection will be as follows:
1. Baseline StiTAs already completed in specific aim #1 to identify major subregions of the M1 forelimb representation: StiTAs at 15 μA during performance of the reach-to-grasp task. Averages will be based on at least 1,000 stimuli.

2. Determination of RL-ICMS evoked final arm posture/hand position: RL-ICMS will be applied at each cortical site. Testing will begin with the hand resting on the home plate device used for the reach-to-grasp task. Initial testing will have been completed as part of specific aim #1. However, in this protocol we will confirm those results and test the responses at additional starting positions of the hand that include the corners of a cube surrounding the RL-ICMS evoked final hand position (orange dots in Fig. 8A) as well as two positions aligned with the shoulder and the RL-ICMS evoked hand position - one closer to the monkey and one further away (blue dots in Fig. 8A). Raisins and other food morsels will be held in the starting locations to prompt the monkey to make movements to those locations. Other locations may also be tested to ensure that the results accurately represent the full work space. Each response will be repeated at least five times to confirm reproducibility and for EMG averaging. Our initial stimulus parameters will be 200 Hz, 30 μA and 500 ms duration. Frequency and duration were chosen to replicate as closely as possible those most commonly used by Graziano et al. (2004). Stimulus intensity will be adjusted to produce a movement that is clear and reproducible, resembling as much as possible, volitional movements in terms of speed and duration (Graziano et al., 2002a).

3. StiTAs at positions straddling the RL-ICMS evoked final hand position: StiTAs at 15 μA will be obtained with the hand at the RL-ICMS evoked final hand position (e.g., central red dot in Fig. 8A) and for positions closer to and further from the monkey (blue dots at the edge of the cube in Fig. 8A.). An isometric push-pull device will be used for this purpose (Fig. 8B). In brief, the monkey will grasp a knob and alternately push and pull on it to generate step changes in force alternating between flexion and extension target zones. Hold periods of one second or longer in each zone will be required. Stimulation at 15 Hz will be applied throughout task performance and averages will be computed separately for push (extension) and pull (flexion). Additional data will be obtained at other positions indicated by the corners of the cube (Fig. 8A, orange dots).

4. StiTAs at the RL-ICMS stimulus intensity: StiTAs as described in #3 above will be repeated but with the stimulus intensity used for RL-ICMS evoked movements. Additional intermediate intensities may be applied to investigate transitions between output effects at 15μA and effects obtained at the intensity used for RL-ICMS evoked movements.

**Measured variables**
The following parameters will be measured for StITA and RL-ICMS output effects:

1. Muscles showing pure facilitation, pure suppression and biphasic effects (usually facilitation followed by suppression). The set of muscles showing stimulation evoked effects is termed the muscle field. The “st” superscript suffix is used to make it clear that this is a muscle field associated with electrical stimulation. Muscle fields will be compared for different types of stimulation and for different stimulation parameters.

2. The magnitude of excitatory effects in averages from StITA and RL-ICMS will be quantified by measuring the peak increase over baseline as a percent of the baseline. The magnitude of inhibitory effects will be quantified by measuring the peak decrease from baseline as a percent of baseline.

3. The latencies of effects will be measured from the onset of the stimulus train for RL-ICMS data or referenced to individual stimuli for StITA data.

4. The final position of the hand in RL-ICMS evoked movements will be identified and confirmed off-line from digital video recordings.

**Interpretation**
We expect to confirm the finding of Graziano et al. (2002a) that output effects from RL-ICMS can change dramatically depending on starting arm posture. On the other hand, we expect that the effects obtained with StITA will be invariant across different arm postures (provided the level of EMG activity is relatively constant), validating the use of this approach for motor output mapping studies. This outcome is expected because of the substantial body of evidence described in the Preliminary Data section supporting the view
that corticospinal output associated with low intensity StTA reflects the activation, either transsynaptically and/or directly, of a local set of corticospinal output neurons; whereas RL-ICMS involves activation of neural circuits more broadly and the output effects from it may not reflect, in any strict fashion, the local organization of corticospinal neurons at the site of stimulation. Disparities between results with StTA and RL-ICMS will suggest that output effects from each type of stimulation involve fundamentally different mechanisms.

Specific aim 2 - experiment 2: isometric wrist task (Figure 9) – joint angle dependent changes in output effects with StTA and RL-ICMS

Data collection and protocol
Data will be collected from at least 20 sites in each of the major forelimb subdivisions of M1 (distal only, proximal only and proximal-distal cofacilitation). Some of these will be the same sites used for experiment #2 – isometric push-pull task.

The data to be collected at each site and the order of collection will be as follows:
1. **Baseline StTAs:** StTAs at 15 μA during performance of the reach-to-grasp task already completed as part of specific aim #1.
2. **StTA at 15 μA during performance of the isometric wrist task.** Five wrist angles will be tested including 0 degrees (hand straight ahead and aligned with wrist), 15 and 35 degrees of flexion and 15 and 35 degrees of extension. For this task, the monkey's hand is held with fingers extended between padded plates and isometric ramp and hold trajectories of force are required alternating between flexion and extension target zones. The gain of the cursor will be adjusted to compensate for expected changes in muscle activity at each position related to the length tension properties of muscle. The goal will be to achieve a similar level of EMG activity across muscles for each wrist angle, i.e., the level of EMG activity at 35 degrees extension will be the same (within limits) as for 35 degrees in flexion. Our current software for StTA will check the EMG activity associated with each stimulus and only accept it if it falls within specified limits.
3. **Repeat #2 but apply RL-ICMS at each joint angle with the monkey at rest (no movement).** A minimum of five trials will be collected.
4. **Repeat #2 but apply RL-ICMS separately during the flexion and extension hold periods at each wrist position.** A minimum of five trials will be collected.

Measured variables
Same as specific aim 2 – experiment 1.

Interpretation
We expect that our results will be quite different than those reported by Graziano et al. (2004). We predict that the sign, magnitude and distribution of StTA effects will not be significantly different at different wrist joint angles. We expect this will be true at the standard 15 μA intensity as well as the RL-ICMS intensity. We will interpret this finding as supporting the view that effects from StTA are the result of activation, either transsynaptically and/or directly, of a local set of corticospinal output neurons that remains relatively invariant with task conditions. We will carefully control background EMG level, which is a measure of motoneuron excitability. This will eliminate effects that may simply be a function of varying proprioceptive input to motoneuron pools related to muscle length. This result will also serve as validation of the StTA method for cortical mapping studies. Predicting the results with RL-ICMS is more problematic. However, we would not be at all surprised if RL-ICMS evoked effects were also invariant with wrist joint angle as long as background motoneuron excitability is controlled. Being sure that background EMG activity is maintained in the presence of RL-ICMS could also be problematic. RL-ICMS tends to interrupt existing active movements (Graziano et al., 2002a). Nevertheless, excitability should be constant, at least for the initial part of the RL-ICMS train. Applying RL-ICMS at different joint angles with the monkey at rest will provide a useful control because in this case we know that motoneuron excitability should vary with joint position. If the predictions
discussed above are confirmed, we will conclude that the joint angle dependence of SITA and RL-ICMS
effects reported by Graziano et al. (2004) can be attributed to large and consistent variations in motoneuron
excitability brought about by joint position dependent changes in muscle spindle input.

**Detailed protocol for specific aim 3**
The goals of this aim are: 1) to test the hypothesis that RL-ICMS evoked “natural” movements result from
the sustained, tonic activity of a broad set of agonist and antagonist muscles whose actions move each joint
to an equilibrium position based on the length-tension relationships of the activated muscles, and 2) to
determine the extent to which EMG patterns associated with RL-ICMS evoked movements are similar to the
EMG patterns associated with the monkey’s own natural, active movements matched to RL-ICMS for
starting and final positions.

**Data collection and protocol:**
1. Monkeys from specific aims 1 & 2 will also be used to collect data for this specific aim. A minimum of 25
sites will be selected for data collection for this aim. The complete maps obtained as part of specific aim 1
will be used to select representative sites from the proximal only and proximal plus distal representations of
forelimb M1.

2. **RL-ICMS evoked movements:** RL-ICMS will be applied to evoke movements from different starting
positions. Final hand position and arm posture will be determined. EMG activity of 24 forelimb muscles will
be recorded on analog tape. Evoked arm movements will be recorded with two digital video cameras for
further offline confirmation and analysis. At each cortical site, RL-ICMS evoked movements will be obtained
from several different starting positions as illustrated in Figure 8 (yellow and blue dots). All RL-ICMS trials
will be repeated a minimum of five times to confirm reproducibility and for averaging. SITA at 15 µA and at
the RL-ICMS intensity will also be obtained to confirm the averages obtained as part of specific aim #1.
Data from specific aims 1 and 2 will also be relevant to analyzing the pattern of EMG activity associated with
RL-ICMS evoked movements and will be used to supplement the new data obtained for this specific aim.
However, the new data will be particularly important because it will have matching EMG records for active
movements between the same starting and ending locations.

3. **Same movement performed actively by the monkey:** After collection of stimulation data is complete, the
monkey will be enticed to actively perform the same movement and with a similar trajectory as the one
evoked by RL-ICMS. This will be accomplished by holding raisins and other food treats at the starting
location. After a hold period of 1-2 seconds, the experimenter will move the raisin through a trajectory
similar to the trajectory of the RL-ICMS evoked movement. When the final hand position of the RL-ICMS
evoked movement is achieved the trial will end with another hold period of 1-2 seconds after which the
monkey will be allowed to each the food morsel. A manually operated TTL reference pulse that is on at the
starting position, off during the movement and back on at the final position will be recorded with the EMG
and video signals and used for generating averages.

**Measured variables**
Averages of EMG activity for 24 muscles will be computed for the RL-ICMS evoked movements and for the
monkey's active, volitional movements using the TTL signal as a reference.

For comparison of stimulation evoked versus active movement EMG patterns, bar diagrams will be
constructed showing which muscles were activated or suppressed during the movement. In addition, the
onset, termination and time of peak activity will be determined for each muscle relative to movement onset
(from the stimulus train or TTL reference signal). Peak magnitude will also be measured. To quantify the
degree of similarity, an index will be developed based on the extent of overlap of activity in the same muscle
for the RL-ICMS evoked movement compared to the monkey's volitional movement. As a further measure of
quantification, we will also cross-correlate the RL-ICMS evoked EMG signal each muscle with the EMG
signal of the same muscle recorded during active movements using methods we have applied in previous
studies (McKieman et al., 2000). This will yield a correlation coefficient from 1 to -1 with 1 being a perfect correlation, 0 being no correlation and -1 being a perfect inverse correlation. A peak correlation of ≥ ± 0.15 will be considered significant. Finally, for RL-ICMS evoked movements, we will inspect EMG records and movement video carefully to confirm that no voluntary responses were superimposed on the later part of stimulation evoked movement.

**Interpretation**

Are the same muscles activated and with similar amplitude and temporal characteristics for RL-ICMS evoked movements compared to the monkey's self-initiated active movements? Substantially dissimilar patterns of EMG activation for RL-ICMS evoked compared to the monkey's own natural active movements will be interpreted as evidence that RL-ICMS does not activate a natural circuit for the movement produced. This will be reinforced by finding that RL-ICMS evoked muscle activity is highly stereotyped with sustained, relatively tonic activation lasting the duration of stimulation. This finding would support the view that muscles activated by RL-ICMS produce joint movements until an equilibrium position is their length tension relationships is achieved. Of course, some modulation in EMG activity might be expected even with RL-ICMS due to changing levels of spindle activity as some muscles shorten and others lengthen.

**Protocol for Specific Aim 4**
The goal of this specific aim is to more fully and systematically document the relationships between ICMS stimulus parameters (intensity, frequency and train duration) and the sign, strength and distribution of output effects evoked with StTA, RS-ICMS and RL-ICMS. We will also determine the extent to which output effects with StTA and other forms of ICMS can be accounted for based on principles of physical stimulus spread in M1 cortex coupled with a detailed knowledge of individual muscle representations.

**Data collection and protocol:**
1. Three new monkeys will be used for these experiments. So the results can be meaningfully interpreted in relation to the known somatotopic subdivisions of the forelimb M1 representation, we will first systematically map at 0.5 mm resolution the spatial extent and magnitude of M1 cortical output with respect to 24 muscles of the forelimb using StTA of EMG activity. This will provide maps of the geometry of each muscles representation in the cortex and contour maps of the strength of output within the representation.

2. Selected sites within each subregion of the M1 forelimb representation will be selected for further study. We will use both the isolated wrist movement task and the reach-to-grasp task. The wrist task provides long periods of relatively stable EMG activity whereas the reach-to-grasp task provides the opportunity to test output to a broader set of muscles. For StTA, stimulus intensity will be varied over a large range (threshold to 150 μA) and changes in the strength and distribution of output effects will be determined. The magnitude of output effects will be plotted against stimulus intensity. StTAs will consist of a minimum of 1000 triggers. A 3-dimensional model of the geometry of the precentral gyrus will be reconstructed from MRIs and layer V maps of individual muscles (based on 15 μA StTA) will be superimposed on this model. The spread of excitatory current through this model tissue volume will be estimated and used to interpret relationships between stimulus intensity and the magnitude of effects in individual muscles and changes in the distribution of effects to different muscles (muscle field) with increasing stimulus intensity.

3. For studies of stimulus frequency and train duration, three representative stimulus intensities will be selected. Ten stimulus pulses will be delivered at three intensities and a wide range of frequencies from 20 Hz to 400 Hz to investigate the effect of frequency. We predict that the magnitude of output effects will plateau before 400 Hz, but if not, we will go to higher frequencies. Dependence on train duration will be investigated using a frequency of 330 Hz. This frequency was selected because of its widespread use in RS-ICMS mapping experiments. The effect of train duration will be investigated with trains of 2 pulses, 3 pulses, 5 pulses 10 pulses, 50 ms, 100ms, 250 ms and 500 ms. We assume that the magnitude of effects will have reached a plateau well before 500 ms, but if not, we will go to longer train durations.
**Measured variables**
The latency and magnitude of stimulus evoked effects will be measured along with the distribution of effects across all 24 recorded muscles. Relationships between magnitude and latency will be plotted against stimulus intensity, stimulus frequency and stimulus train duration.

**Interpretation**
A 3-dimensional model of the geometry of the precentral gyrus will be constructed from MRIs and layer V maps of individual muscles (based on 15 μA StTA) will be superimposed on this model. The spread of excitatory current through this hypothetical tissue volume will be modeled and used to interpret relationships between stimulus intensity and the magnitude of effects in individual muscles as well as changes in the distribution of effects to different muscles (muscle field) with increasing stimulus intensity. The data obtained from these experiments will provide a stronger foundation for the use of ICMS in cortical mapping. The modeling studies will also provide a test of the extent to which the magnitude and distribution of effects to different forelimb muscles is consistent with simple physical spread of current through the cortical tissue volume.

**Feasibility and Potential Problems – All Specific Aims**
We have experience with nearly all the behavioral, electrophysiological and imaging methods proposed and do not anticipate any difficulties. The one exception is the arm push-pull task. We will probably fabricate the isometric push-pull device by modifying a Grass Instruments strain gauge transducer. This should only require a relatively simple modification and the output will adapt to our existing behavioral control paradigm.

One area in which we will need help is 3-D modeling of the precentral gyrus with embedded maps of individual muscles. As part of this effort, we also propose to model the distribution of excited layer V corticospinal neurons based on physical current spread. Of course physiological (synaptic spread) could significantly enlarge the distribution of excited corticospinal neurons. Nevertheless, we would like to determine the extent to which a detailed knowledge of the location and geometry of individual muscles maps based on 15 μA StTA, coupled with models of current spread, might predict expansion of the muscle distribution and the strength of effects in StTAs as stimulus intensity is increased. Another issue is whether the output effects from RS-ICMS and RL-ICMS can be predicted in the same way. With repetitive ICMS methods we anticipate much greater deviation from prediction based on modeling because of greater spread due to temporal summation.

**Methods Common to All Specific Aims**

**Behavioral tasks**

*Reach-to-grasp:*
This task has been described previously (Belhaj-Salif et al. 1998; McKiernan et al. 1998). Reach-to-grasp requires coactivation of multiple proximal and distal forelimb muscles in natural, functional synergies. During each data collection session, the monkey is seated in a custom primate chair, and placed inside a sound-attenuating chamber. The limb not being used for task performance is restrained. The reach-to-grasp task consists of four phases as illustrated in Figure 7. Performance is guided by computer generated audio and video cues.
**Isometric arm push-pull task:**

A. Figure 8. A. For each cortical site, the final hand position (arm posture) associated with RL-ICMS will be found (e.g., red dot). Movement to this final position independent of starting positions (yellow and blue dots) will be confirmed. Two starting positions (blue dots) will then be used to test output effects on EMG activity of 24 muscles with SITA, RS-ICMS and RL-ICMS during performance of an isometric push-pull task.

B. The isometric task will require gripping a knob and making alternating push (extension) and pull (flexion) movements as illustrated. The design will prevent forces other than those aligned with the long axis of the connecting rod to be transduced. This will be accomplished by running the connecting rod through a bushing in the wall of the transducer box.

**Isometric wrist task:**

Figure 9. Illustration of isometric wrist task. The monkeys lower and upper arm are restrained. The hand with fingers extended are placed in a manipulandum that rotates about the wrist. The manipulandum can be locked into a fixed position at any degree of rotation between 90 degrees of flexion and 90 degrees of extension. Joint angle is transduced with a precision potentiometer and wrist torque is transduced with a load cell. The load cell is mounted between the manipulandum and a brake that allows the manipulandum to be fixed at specific angles of joint rotation. The monkey must generate ramp and hold trajectories of wrist torque alternately between flexion and extension. Also shown are EMG records from forearm wrist and digit muscles and the activity of a simultaneously recorded cortical neuron.
Cortical implant and surgical methods
On completion of training, each monkey will be implanted with a cortical recording chamber. For all implant surgeries, the monkeys will be tranquilized with ketamine (10 mg/kg), administered atropine, and subsequently anesthetized with isoflurane gas. Monkeys will receive prophylactic antibiotic before and after surgery and analgesic medication postoperatively. All surgeries will be performed in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care using full sterile procedures. All procedures will conform to the Guide for the Care and Use of Laboratory Animals, published by the United States Department of Health and Human Services.

A magnetic resonance imaging (MRI) compatible plastic chamber allowing exploration of a 30-mm-diameter cortical area will be stereotactically positioned over M1 cortex and the premotor forelimb area of the left hemisphere of each monkey using procedures fully described previously (Kasser and Cheney 1985; McKiernan et al. 1998). For MRI compatibility, titanium screws and titanium restraining nuts will be used. In addition, a titanium screw in contact with the dura will serve as a reference ground for electrophysiology.

EMG implant methods
EMG activity from 24 muscles of the forelimb will be recorded using pairs of multi-stranded stainless steel wires implanted during a separate sterile surgical operation. Our procedures for implanting forelimb muscles are fully described elsewhere (Park et al., 2000). Briefly, pairs of wires for each muscle are tunneled subcutaneously to their target muscles. In one type of implant procedure (modular subcutaneous implant) four connector modules are placed on the upper arm. Two connectors supply the wrist and digit muscles, one supplies the elbow muscles and one supplies the shoulder muscles. The connectors and any exposed wire is then anchored to the arm with elastomeric tape and the monkey wears a jacket to protect the implant. A variation of this method involves channeling all EMG wires subcutaneously to a circular connector anchored to the skull near the cortical recording chamber. Although the implant procedure is longer and more difficult, this procedure has the advantage that no protective jacket is required. The wire insertion points for specific muscles are identified on the basis of external landmarks and palpation of muscle bellies. The wires of each pair are bare at the tip and inserted into the muscle with a separation of ~ 5 mm. Proper placement is tested by stimulating through the wires with short stimulus trains while observing the evoked movements. If proper placement is not confirmed, the wires are removed and reinserted.

EMGs will be recorded from five shoulder muscles: pectoralis major (PEC), anterior deltoid (ADE), posterior deltoid (PDE), teres major (TMJ), and latissimus dorsi (LAT); seven elbow muscles: biceps short head (BIS), biceps long head (BIL), brachialis (BRA), brachioradialis (BR), triceps long head (TLON), triceps lateral head (TLAT), and dorso-epitrochlearis (DE); five wrist muscles: extensor carpi radialis (ECR), extensor carpi ulnaris (ECU), flexor carpi radialis (FCR), flexor carpi ulnaris (FCU), and palmaris longus (PL); five digit muscles: extensor digitorum communis (EDC), extensor digitorum 2 and 3 (ED23), extensor digitorum 4 and 5 (ED45), flexor digitorum superficialis (FDS), and flexor digitorum profundus (FDP); and two intrinsic hand muscles: abductor pollicis brevis (APB) and first dorsal interosseus (FDI). At regular intervals, the monkeys will be tranquilized with ketamine and the implants retested to confirm electrode location.

Recording and stimulation methods
Glass and mylar insulated platinum-iridium electrodes with impedances between 0.7-1.5 MΩ will be used for cortical recording and stimulation. Electrode penetrations will be made systematically in precentral and postcentral cortex using a 0.5 mm grid interval. In the bank of the precentral gyrus, the electrode will be advanced with a manual hydraulic microdrive. Stimulation will be performed at 0.5 mm intervals, starting from the first cortical electrical activity encountered. Cortical
electrical activity and EMG activity will be monitored simultaneously along with task-related signals. All original data signals will be saved on a TEAC 32 channel instrumentation tape recorder.

During task performance, stimuli (15-150 μA at less than 20 Hz) will be applied through the microelectrode and will serve as triggers for computing STTAs. Individual stimuli are symmetrical biphasic pulses - a 0.2 ms negative pulse followed by a 0.2 ms positive pulse generated by a Grass S88 stimulator and a pair of stimulus isolations units.

EMGs will be digitized at a rate of 4 kHz, and STTAs compiled over a 60 ms epoch, including 20 ms before the trigger to 40 ms after the trigger. Assessment of effects is based on STTAs of at least 1000 trigger events. Segments of EMG activity associated with each stimulus are automatically evaluated within the data acquisition software and accepted for averaging only if adequate EMG activity is present. As a general procedure, the average of all EMG data points over the entire 60 ms epoch must be equal to or greater than 5% of the full-scale signal. This avoids averaging segments in which EMG activity was minimal or absent (McKiernan et al. 1998). For the experiments of specific aim 2, this feature will be set as window to ensure that the level of EMG activity is similar at different joint angles.

Repetitive ICMS (R-ICMS) will consist of short and long train durations designated RS-ICMS and RL-ICMS respectively. RS-ICMS is a traditional method and consists of a train of 10 symmetrical biphasic stimulus pulses (negative—positive with total duration of 0.4 ms) at a frequency of 330 Hz (Asanuma and Rosén 1972). RL-ICMS will parallel the work of Graziano and colleagues with a train duration of about 500 ms. For RL-ICMS, stimulus intensities will be in the range used by Graziano et al. (2002a). Intensity will be raised until movements with natural velocities and clear final positions are observed. Averages of the RS-ICMS and RL-ICMS trials will be computed using an epoch of about one second. The first stimulus in the train will serve as the trigger.

**Magnetic resonance imaging (MRI) of cortex and analysis**

MRIs will be obtained to identify the location and gyral pattern of the frontal lobe. This data will be used to confirm electrode track locations as we have done in the past (Park et al., 2001). Imaging will be performed in a new stand alone imaging building containing 3T and 9.4T systems. We have done several MRIs on the 9.4T system and intend to use this system for the work proposed. The high quality of the 9.4T images will be particularly valuable for specific aim 4. The monkeys will be tranquilized with ketamine and atropine and subsequently anesthetized with isoflurane gas. A custom-designed chamber cap filled with an MR opaque marker (liquid vitamin E) will be used to identify the anterior–posterior (A-P) and medial–lateral (M-L) axes of the cortical recording chamber. Image reconstruction and analysis will be performed using Omniview 2D and 3D visualization software [Private Source] the details of which have been described previously (Park et al. 2001). A series of oblique parasagittal images of the cortex will be obtained with respect to the recording chamber coordinate system. These images will be orthogonal to the M-L axis and in register with the chamber coordinate system. Thus, the images will be parallel to a series of electrode tracks having the same M-L coordinate. For example, an oblique parasagittal image at lateral 4 would represent a slice through the cortex showing all electrode tracks for which the M-L chamber coordinate was lateral 4. These images will then be traced to highlight gray matter, white matter and the central sulcus. From these images, we will estimate electrode track and stimulation site placement.

**Data analysis**

At each stimulation site, averages of EMG activity from 24 muscles will be obtained. Poststimulus facilitation (PSF) and suppression (PSSt) effects will be computer measured as described in detail by Mewes and Cheney (1991). First, nonstationary, ramping baseline activity will be routinely subtracted from the average using custom data analysis software. Mean baseline activity and standard deviation (SD) will be measured for each average in the pretrigger period. Effects in STTAs will be considered significant effect if the envelope of the STTA exceeds ±2 SD of baseline for a period greater than or equal to 0.75 ms (3 points). The onset latency of PSF and PSSt effects will be measured as the point where the envelope of
the effect intersects the two standard deviation line. The magnitude of PSTF and PSTS will be expressed as the percent increase or decrease in EMG activity above (facilitation) or below (suppression) baseline (Cheney and Fetz 1985; Cheney et al. 1991; Kasser and Cheney 1985). Peak values will be measured as the highest point in the peak of facilitation or lowest point in the trough of suppression. We will also measure magnitudes as signal-to-noise ratios (peak or trough minus baseline divided by baseline standard deviation). Signal-to-noise measures are not influenced by changes in baseline EMG activity. All magnitudes based on signal-to-noise ratio were normalized to 2,000 trigger events using the principle of signal averaging that baseline noise should decrease as the square root of the number of trigger events (Belhaj-Saïf et al. 1998).

_Undertaking the cortex to make 2-D maps_

The procedure we will use for creating a two dimensional map based on MRI and electrophysiological data is described in detail in Park et al. (2001). White matter will be identified by a sharp decrease or loss of background cell activity. Sensory cortex will be identified by the presence of distinctive spike activity and characteristic receptive fields (Widener and Cheney 1997). For each electrode track, sites corresponding to cortical layer V will be identified using a combination of electrode depth, strength of PSTF effects, and reconstruction of precentral geometry in relation to MRI sections. Electrode penetrations on the convexity of the gyrus traverse cortical layers perpendicularly, and in these cases, it is relatively easy to identify the stimulation site closest to layer V. For electrode penetrations traversing the depth of the precentral gyrus and extending roughly parallel to the cortical layers, it is more difficult to identify layer V sites. In these cases, output effects from sites at the same depth from different electrode tracks along the same A-P axis will be compared to aid in identifying sites most likely corresponding to layer V.

_Statistical analysis_

Evaluating the significance of post-stimulus effects will follow procedures we have used previously (Park et al, 2004). Segments of the record following the trigger that rise about two standard deviations of the baseline points. The significance of these points is determined using a t-test and comparing pre-trigger data points with a similar period following the trigger. This approach will be applied to effects in records from SITA, RS-ICMS and RL-ICMS. Evaluation of arm posture and joint angle changes in the magnitude of output to 24 muscles of the forelimb will be based on ANOVA with a Mann-Whitney test.

_Timetable of experiments_

Year 1: Identify and purchase three rhesus macaque monkeys, complete quarantine requirements (minimum three months), begin training (9-12 months depending on the monkey's age), implement the isometric push-pull task.

Year 2: Complete training on three monkeys and begin data collection and analysis for specific aims 1-3.

Year 3: Complete data collection and analysis for specific aims 1-3. Identify and purchase three rhesus macaque monkeys for specific aim 4 and begin training on tasks.

Year 4: Complete papers for specific aims 1-3. Complete training for specific aim 4 and begin data collection.

Year 5: Complete data collection and analysis for specific aim 4. Complete publication of manuscripts.
E. Human Subjects
None.

F. Vertebrate Animals

1. Description of Procedures:
We propose to use a total of 6 adolescent male rhesus monkeys (Macaca mulatta) for this project over a period of 5 years. The monkeys will weigh 3-4 Kg at the time of purchase and we will work with each of them for periods ranging from 2-4 years.

Housing:
Monkeys will be housed in a fully AAALAC accredited Animal Care facility and transported daily to the laboratory for training or recording.

Behavioral procedures:
Monkeys are seated in a primate chair adjusted carefully to match their height. The monkey wears a nylon collar that attaches to the chair's neck plate. Movement in and out of the home cage is facilitated by use of the collar and pole method. Monkeys become quite cooperative in moving from the cage to the chair. The primate chair is covered for transport of the monkeys to the laboratory. All procedures in the laboratory are performed in a large (8' X 8' X 7') sound attenuating steel chamber. One of the monkey's arms is restrained loosely in a padded tube; the other arm is free to operate various movement task devices. In all cases, arm restraining devices are fitted to the arm and padded with foam. For this proposal, monkeys will be trained on three forelimb tasks. The forelimb tasks consist of an isometric step tracking wrist movement task, a task requiring the monkey to grasp a handle and make push-pull movements of the arm, and a reach-to-grasp task. Successful performance of tasks is rewarded with applesauce and food pellets. The food pellets are designed to be a complete diet. Only positive reinforcers are used. Once trained, monkeys will generally work continuously for 2-4 hours per day depending on the task.

MRI procedures:
Before implanting the recording chamber and EMG electrodes, monkeys will be tranquilized with ketamine and transported for acquisition of initial structural MRIs. Both a Siemens head only 3T system and a Varian 9.4T system are available for this purpose. We will use the 9.4T system. Monkeys are covered with a blanket and heart rate and blood oxygenation are monitored with a Doppler system. We also give the monkeys ear protection during this procedure. The procedure is complete in an hour and the monkeys are monitored until sitting up in their home cage and taking food. Another MRI will be obtained after attaching the recording chamber.

Recording chamber implants:
Once training is complete, an MRI compatible chamber will be attached to the skull under full sterile conditions and isoflurane anesthesia. Monkeys are initially tranquilized with ketamine and atropine and transported to the surgery facility. For implantation, the bone under the chamber is removed but the dura is left intact. The chamber is positioned and fixed to the skull with titanium screws and dental acrylic. For stability and chamber placement, the monkey's head is placed in a stereotaxic apparatus after a surgical level of anesthesia is obtained. The head is held by ear bars in the external ear canals and by support bars on the upper jaw and the lower orbit of the eye. During all surgeries, the eyelids are held shut with ophthalmic ointment and the following vital signs are monitored: 1) blood oxygenation, 2) heart rate, 3) respiration, and 4) rectal temperature. For surgeries lasting more than 3 hours, Ringer's solution is administered as a slow drip through an i.v. line in the femoral vein. For recovery procedures see section on "procedures to minimize pain".
**EMG electrode Implants:**
These experiments require recording the electrical activity from 24 muscles of the forelimb including shoulder, elbow, wrist, digit and intrinsic hand muscles. Separate sets of connectors and wires are used for each of these muscle groups. A pair of multi-stranded stainless steel wires[Private Source] is inserted into each muscle using a 22 gauge hypodermic needle. Wire bundles to each muscle group are tunneled under the skin from and entry point on the upper arm. Tunneling is accomplished with hypodermic needle stock, cut to appropriate lengths and sharpened on one end. For insertion into muscles, wires are back fed into the tip of the needle and inserted in the muscle through puncture openings in the skin made with a number 11 scalpel blade. The needle is then withdrawn leaving the wire with a hooked end anchored in the muscle. Proper location of electrodes is confirmed by observing appropriate movements with electrical stimulation. Electrode exit points on the upper arm (again, only puncture openings) are anchored with medical elastomeric tape and protected by a custom made nylon vest that the monkey wears in its home cage. These EMG implants are well tolerated by the monkey and remain functional for periods of 2-5 months. Monkeys are generally implanted with EMG electrodes 4-6 times over a period of 2-4 years. However, this procedure is relatively non-traumatic. There is essentially no blood loss with this procedure. The EMG implant requires about seven hours to install and does not involve entering a major body cavity. Monkeys are able to work on behavioral tasks immediately upon recovery from anesthesia. Monkeys are given 3-4 weeks of recovery after an implant is removed. Implants longevity depends largely on attention to carefully maintaining the wire exit points on the upper arm.

**Recording procedures:**
While the monkey is performing movements, a microelectrode is advanced through the dura, into the brain, for recording the action potential discharges of single neurons. These procedures are performed with the monkey fully awake and performing the task. Judging from the fact that electrode and cannula insertion (if necessary) into the brain does not interrupt the monkey's performance, none of these procedures seem to be particularly uncomfortable. This is not surprising in view of the fact that actual surgical removal by suction of parts of the brain in humans for intractable epilepsy is not painful and is performed with the patient fully awake.

**Justification for multiple surgeries:**
Multiple major surgeries defined as multiple entries into a major body cavity are not planned in this proposal. EMG electrodes will be implanted multiple times in each monkey. However, as discussed above, these implants are not traumatic and are not considered major surgeries.

**2a. Justification for Use of Animals:**
The objectives of this research could not be achieved without the use of animals. The questions we are attempting to answer concern the functioning of the intact motor system during voluntary movement. Monkeys are used because of the similarity of their brains to the human brain in terms of mechanisms involved in the control of movement. Invertebrates are inappropriate for these studies because the organization of motor function in the invertebrate nervous system is fundamentally different than in vertebrates. For example, invertebrates lack a cerebral cortex and cerebellum - two very important structures for motor control in mammals. Tissue culture systems are inadequate because they fragment the brain's motor system, precluding any possibility of investigating meaningful interactions of populations of neurons as an complete system. Mathematical models can be helpful as an adjunct to these experiments but not as a replacement for them because, to date, it has been difficult to adequately model even the most accessible and simple parts of this system. Our level of fundamental knowledge is simply too incomplete to predict the functioning of the system under a wide range of circumstances and manipulations without investigating those situations directly.

**2b. Justification for Number of Animals to be Used:**
We propose to use a total of 6 adolescent male rhesus monkeys (Macaca mulatta) for this project over a period of five years. Each monkey will be used for a period of about 2-4 years. This number of monkeys is
based on past experience concerning the yield of data from individual monkeys and the need to replicate results in more than one animal. A minimum of two monkeys is needed for each project. Three are requested so we have one backup. It should be emphasized that use of chronically implanted animals is designed to maximize the amount of information that can be obtained from one animal, thereby minimizing the number of animals needed.

3. Veterinary Care:
Maintenance and care of our monkeys is supervised by three veterinarians - one with certification in laboratory animal medicine and another working toward this goal.

4. Procedures to Minimize Discomfort and Injury:
All surgeries will be performed in an approved facility under antiseptic, sterile conditions. Surgeries for implantation of chambers and EMG electrodes will be performed under surgical level isofluroxane anesthesia. All monkeys will be carefully monitored after anesthesia until fully awake and eating. Postoperative care consists of closely monitoring the monkey until it is fully awake and able to sit and stand without assistance. Rhesus monkeys recover quickly following gas anesthesia and usually begin eating within an hour of awakening. Postoperative analgesics will be given by the veterinary staff once the monkey is fully awake. Antibiotics will be used to control infections that might develop, although this has rarely been a serious problem. Nevertheless, wound edges will be inspected daily and treated with Betadine and topical antibiotic. Microelectrodes will be inserted into the brain while the monkey is awake. However, these procedures do not appear to produce discomfort because the monkey continues to work uninterrupted while the electrode is advanced into the brain.

5. Euthanasia Methods:
For euthanasia, the monkey will first be tranquilized with Ketamine and then given an overdose of barbiturate administered intravenously. This is consistent with recommendations of the Panel on Euthanasia of the American Veterinary Medical Association.

G. Literature Cited


Principal Investigator/Program Director (Last, First, Middle):  
Cheney, Paul D.

H. Consortium/Contractual Arrangements  
None

I. Letters of support  
None
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. ☐ SBIR Fast Track
☐ STTR Phase I  ☐ STTR Phase II: STTR Phase I Grant No. ☐ STTR Fast Track

☐ REVISION of application number:
(This application replaces a prior unfunded version of a new, competing continuation, or supplemental application.)

☐ COMPETING CONTINUATION of grant number:
(This application is to extend a funded grant beyond its current project period.)
☐ No ☐ Previously reported

☐ SUPPLEMENT to grant number:
(This application is for additional funds to supplement a currently funded grant.)
☐ Yes. If "Yes," ☐ Not previously reported

☐ CHANGE of 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).

<table>
<thead>
<tr>
<th>Budget Period</th>
<th>Anticipated Amount</th>
<th>Source(s)</th>
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<td></td>
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2. ASSURANCES/CERTIFICATIONS (See instructions.)
The following assurances/certifications are made and verified by the signature of the Official Signing for 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.

- Human Subjects
- Research Using Human Embryonic Stem Cells
- Research on Transplantation of Human Fetal Tissue
- Women and Minority Inclusion Policy
- Inclusion of Children Policy
- Vertebrate Animals

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

☒ DHHS Agreement dated: 07/09/02 ☐ 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 $250,000 x Rate applied 47 % = F&A costs $117,500
b. 02 year Amount of base $250,000 x Rate applied 47 % = F&A costs $117,500
c. 03 year Amount of base $250,000 x Rate applied 47 % = F&A costs $117,500
d. 04 year Amount of base $250,000 x Rate applied 47 % = F&A costs $117,500
e. 05 year Amount of base $250,000 x Rate applied 47 % = F&A costs $117,500

TOTAL F&A Costs $587,500

*Check appropriate box(es):
☐ Salary and wages base ☒ Modified total direct cost base ☐ Other base (Explain)
☐ Off-site, other special rate, or more than one rate involved (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.)

PHS 398 (Rev. 05/01)