

AGENDA

ANIMAL USAGE FORM

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PR1 07/17/07

IACUC Use Only			
IACUC Study #	0707A11902	Approved:	9/7/07
IACUC Chair:	Tom Molitor	RAR Veterinarian:	Cynthia S. Gillett
Approval Duration:			

Part A

**0. Project Identification and Signatures**

0A. Type of Application:  New Protocol  3-year Renewal of IACUC #0407A61882  
(If this is a 3-year renewal, do not use language referring to the previous protocol or grant in this form.)

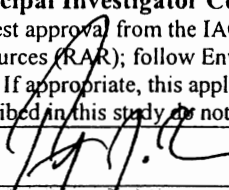
Anticipated Starting Date: September 5, 2007

0B. Project Title: (Project title must match grant title. If different, also provide grant title)

Optical Monitoring of Cortical Neuronal Activity in the Behaving Primate.

0C. Is this an Agricultural Project? (Use of agricultural animals in non-biomedical research)  Yes.  No.

0D. Principal Investigator (Must be faculty or academic professional administrative staff.)

Name (Last name, First name MI): <b>Ebner, Timothy J.</b>	Phone Number: 612-626-2205
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Occupational Position: <input checked="" type="checkbox"/> Faculty <input type="checkbox"/> Staff (must be P & A) <i>Note: students cannot be principal investigator.</i>	University Department (if applicable): Neuroscience
<b>Principal Investigator Certification:</b> If the IACUC approves my application, I agree to execute this work as described; request approval from the IACUC for changes; comply with the guidelines set forth by the IACUC and Research Animal Resources (RAR); follow Environmental Health and Safety guidelines; and be responsible for the supervision and work of my staff. If appropriate, this application accurately and completely reflects the animal use in the full grant application. The activities described in this study do not unnecessarily duplicate previous experiments.	
	Professor and Dept Head
Original Signature of PI	Title of PI
	Date: 7/2/07

If PI is not a University of Minnesota faculty member, IACUC may notify you that additional signatures will be required.

0E. Person preparing this document

Name: Kris Bettin Phone number: 612-626-2205 Email: betti002@umn.edu

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### 3. Specific Aims & Details of Animal Use

#### 3A. What is the goal/specific aim of this project? What is the research or development question?

Describe the relevance of the study to advancing scientific knowledge and/or the benefits of the study to human and/or animal health. Provide sufficient information to indicate that the potential new knowledge from the project justifies the use of animals. Jargon should be avoided or explicitly explained (please define all acronyms).

In the mammalian brain, the dorsal premotor cortex (PMd) plays an important role in the transformation of visual information into motor commands from the primary motor cortex (M1). From chronic single unit recording studies, it is known that the firing of neurons in the PMd is directionally tuned (i.e., they fire preferentially for certain directions of arm movement). This directional processing is important to the mapping of visual target locations to the direction of arm movements. The distribution of directional tuning over the PMd cortex has been approached using chronic single unit recordings in several labs; however, single unit recordings have not permitted a determination of whether direction is mapped spatially on the motor cortices.

The first specific aim is to demonstrate that optical imaging can be used to map cortical activity in the behaving primate. We have developed two new optical imaging methodologies in our laboratory. The first monitors an intrinsic signal based on flavoprotein autofluorescence. The second is based on the nontoxic pH dye neutral red. We propose to compare the neutral red optical signals with flavoprotein autofluorescence signals while a trained monkey completes a circular tracking task. Optical imaging can provide high-resolution maps of the underlying brain activity. After mounting a specialized optical chamber over the motor cortical areas. A high speed, high sensitivity camera system will be coupled to the optical chamber fixed over the cortex or primate chair. Images of the brain activity will be taken during intracortical microstimulation (ICMS) using short and long duration stimulation protocols. Imaged activity will be used to determine topographical and functional mapping of the cortical area in addition to cortical activity patterns and EMG responses to stimulation amplitude, frequency, and duration.

The second specific aim is to determine how the direction, amplitude, and speed of visually moving targets and the direction of hand movements during target tracking are spatially mapped in the PMd. The task has both a visual only component and a component with movement of the hand. For each target movement direction (displayed in increments of 45° on a vertically positioned computer monitor in front of the monkey) an exposure of the camera system is triggered. Areas of activity over the PMd and M1 as a function of the target movement direction are thus mapped. The aims are accomplished using a behavioral paradigm already well studied by this lab using conventional single unit electrophysiological techniques.

Overall, these studies will provide insight as to: 1) whether intrinsically based flavoprotein autofluorescence and/or neutral red optical signals are feasible techniques for imaging brain activity in behaving primates, 2) identify topographical and functional mapping of arm/hand areas during ICMS in PMd and M1, and, 3) how movement direction is mapped over the PMd and M1 motor cortices, and how this mapping changes with sensorimotor behavior (i.e., between visualization of direction and movement direction).

#### 3B. If this application is a continuation of an ongoing project, please state concisely how these goals differ from those in the original application and what was accomplished during the prior approval period.

This study proposes to first determine if flavoprotein autofluorescence and/or neutral red imaging is optimal for monitoring brain activity in the behaving primate. Using flavoprotein autofluorescence imaging in a preliminary study we were able to demonstrate the feasibility of the technique and that potential problems, including infection and seizures, could be effectively managed. However, the NHP used in the study had health issues unrelated to the chamber implant (i.e., high blood pressure and stroke) that did not allow for the completion of the study. Specifically, while data was successfully

collected across a broad band of signal wavelengths, more targeted wavelength recordings were not completed. The targeted wavelength recordings are necessary for showing not only the source of the signal but also to increase the signal-to-noise ratio of the imaged area for accurate topographical and functional mapping.

**3C. Provide a complete and accurate description of what procedures will be performed on/with the animals. Answer in lay language or language understood by a person unfamiliar with your area of research (*define all acronyms*). Jargon should be avoided or explicitly explained. *Do not cut and paste from a grant proposal or include language or explanations that are not relevant to animal use.***

- Provide sufficient detail to allow evaluation by the IACUC. You are strongly encouraged to use a diagram or chart to explain complex designs. **(Use additional pages if needed)**
  - Describe all procedures, their frequency and time points over the course of the experiments. Be certain to detail the pain classification of each animal group. This should correspond to the information you provided in the **Animal Request Table** (Section 1).
  - Include how long the animals will be maintained. Include dose, route of administration and frequency of any drugs to be administered.
  - Describe methods used in behavior studies (including use of noxious stimuli or other methods of positive or negative reinforcement).
  - Surgery should be described here only as it relates to the study design. Surgical details should be provided in Appendix F.
  - For animals used in agricultural projects, you may reference the study code number of the IACUC approved Standard Operating Procedures for the housing facility and husbandry, as applicable.
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These optical imaging studies will parallel established methods used for chronic single cell recordings in the primary motor cortex in behaving primates. For specific aim 1, monkeys are trained to sit in a primate chair and place their hand in an initial start position prior to intracortical microstimulation and optical imaging of the motor cortex. For specific aim 2, each animal is trained over a period of 6-12 months to perform controlled, visually guided arm and hand movements to track a moving target displayed on a computer monitor. After successful training, optical imaging of the motor cortex is done while the animal completes the tracking task. In an initial surgery a head restraint system is implanted on the skull. Once trained, chronic recording hardware is surgically placed on the skull.

**Animal Training:** The animals are trained to sit in a primate chair, reach to and grasp a manipulandum and track a moving target (linear, circular, or random pattern). Once the monkey is able to perform this task within the timing and tracking requirements of the task, posts are implanted in the skull so that the animals can be further trained to complete the task while their heads are restrained. Water is used as a reward for the animal to learn and execute this complex behavioral task.

**Water restriction:** As noted in section J5, strict operating procedures (developed in collaboration with RAR) are followed with regard to the water restriction of NHPs. The goal is to acclimate the animal to a baseline level of water without harm or injury. Water restriction is necessary for the animals to complete their tasks with fluid as a reward. The SOP outlined in section J5 also provides specific instructions, fluid intake levels, allowable weight fluctuations, and duration over which the acclimation to a baseline intake level should take place. Furthermore, the monitoring of these animals' health is done in close collaboration with the RAR veterinarians and animal care staff, including weekly weigh-ins, daily assessment of the animal's behavior (activity level, arousal, performance in the testing, interaction with investigators and staff) and skin coat (luster, color, etc), as well as daily logs of water intake. The SOP detailed in section F5 also specifies procedures for water rations before and after surgeries, for young animals, and staff vacation periods.

**MRI Procedure:** While brain atlases and experience from prior surgeries on other animals are generally consulted for targeting the desired recording area of the brain, on occasion the natural variations in the brain anatomy across the animals may result in poor accuracy. As an added precaution, performing an

MRI scan on some animals prior to the surgery may be needed to increase accuracy. The procedure itself is noninvasive and can be considered diagnostic. The MRI scans enable us to better target the desired brain areas for individual animals when surgically placing the recording chamber. The schedule for completing MRI scans is further detailed in section F6.

Data Collection: Once the animal has gained proficiency in completing the tracking task demands and the recording chamber has been implanted in the skull (6-12 months), data collection can be done. This consists of a cylindrical chamber fastened over a trephine hole in the skull and several points of fixation for a halo for head immobilization. The major point of divergence with the present work in our laboratory is that the dura within the chamber is replaced with a transparent artificial dura. Antibiotics are given to prevent infections and, if necessary, dilantin is given to prevent seizures. After implantation of the chamber the cortical activity will be studied using optical recording techniques. A camera system is coupled to the chamber mounted on the head or the primate chair. For the autofluorescence signal experiments, the optical signals are monitored during intracortical microstimulation and/or while the monkey is performing the tracking task. For the neutral red experiments, the dye (5-8 mM) will be infused into the chamber to stain the brain and the optical signals will be monitored as the animal performs the task. Single unit recordings of motor cortex neurons using chamber access to the brain will be on a daily basis. In some of the recordings, a microelectrode will be introduced to record the activity of single neurons. Simultaneous electromyographic (EMG) activity of arm and shoulder muscles is recorded using needle electrodes. Non-invasive systems such as video-based recording are used to monitor limb and/or eye movements. On occasion, it may be necessary to clear the cortex of fibrous growth that may occlude the imaging field. This procedure will require the use of a microscope and fine forceps to remove the microscopic fibers, thus requiring the animal to be lightly sedated during the procedure. Once the area of the brain accessible through the chamber is fully mapped and/or excessive fiber growth cannot be cleared, the animal must then be re-trained to perform the task using the other hand. After successful retraining, the original head chamber is removed, all wounds allowed to heal and a second chamber is placed on the opposite site of the head. Optical recordings in the second hemisphere of the brain will be done as above.

Potentially, a third chamber is placed. In our previous experience, moving the chronic unit recording chamber can be accomplished with no increased risk or trauma to the animal beyond that of a single surgical procedure. Also, the duration of the successive surgeries is considerably shorter since the restraining hardware is already in place. Second and third chamber placements would be performed only if the animal is in excellent health and it is highly likely that there would be a substantial increase in the results obtained from the animal. The additional survival surgeries are needed in order to meet the data collection requirements for publication (see sections 3D and 6A), to minimize the number of animals used, and to increase the efficiency and productivity of the experimental protocol (see section F11).

Euthanasia and Histology: At the final stage of the study the animal is sacrificed for histological processing of the brain to determine recording locations. End point of the study is when the imaged area has been mapped and/or excessive fibrous growth within the imaged area can no longer be cleaned. Animals are maintained for 2-3 years. In some cases, a non-survival surgery will precede euthanasia in order to image a more extensive cortical area than that available with the recording chamber.

**3D. For each species listed on the “Animal Request Table” in section 1, list your experimental and control groups. Indicate the number of animals in each and to which pain classification (A, B or C) they belong (a table format is highly recommended).** The number of animals must add up to the total number of animals requested in section 1 and, if applicable, those discussed in Appendix B (breeding). This response should correspond to the response in question 3C.

Each of the two specific aims requires imaging of the cortex in 4 monkeys. Therefore, we are requesting a total of 8 animals on this protocol as follows:

Specific Aim 1: 4 monkeys (3 currently in-house, to be transferred; 1 to be purchased)  
Specific Aim 2: 4 monkeys (4 to be purchased)