

ANIMAL USAGE FORM

AGENDA

Version 2.51
Updated 26 Aug 2004

FRI 5/22/07

IACUC Use Only			
IACUC Study #	0705A07821	Approved:	7/11/07
IACUC Chair:	Tom Malotka	RAR Veterinarian:	Gyathia S. Gilbert

05-09-07P01:49 RCVD

Part A

0. Project Identification and Signatures

0A. Type of Application: New Protocol 3-year Renewal of IACUC #0407A61885
(If this is a 3-year renewal, do not use language referring to the previous protocol or grant in this form.)

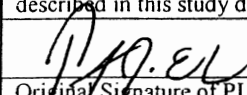
Anticipated Starting Date: July 11, 2007

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

**Role of the Cerebellum in Visually Guided Arm Movements.
Encoding Reach and Grasp in Cerebellar Neuronal Activity**

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): Ebner, Timothy J.	Phone Number: 612-626-2205
Mailing Address: 2001 Sixth Street SE 421 Lions Research Bldg Minneapolis, MN 55433	Pager or Cell Phone Number:
U of M Employee ID: 0728223	Fax: 612-626-9201
U of M x.500 ID (ex. smith001): ebner001	Email: ebner001@umn.edu
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
Principal Investigator Certification: 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: 5/8/07

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

CIRCULATION COPY

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).

This grant proposal tests the global hypothesis that the cerebellum is the site of both forward and inverse internal models of the arm. Internal models provide for representations of the input-output properties of the motor apparatus or their inverses. A forward model predicts the state of system, either the motor variables or the sensory output, as a consequence of the current state of the arm and the motor commands. An inverse dynamics model transforms the desired trajectory into the torques and forces needed to control the arm. The results of numerous psychophysical studies support the hypothesis that the central nervous system utilizes internal models to control movements. Although widely hypothesized that the cerebellum acquires and stores internal models of the arm, there are few explicit tests of this hypothesis based on single cell recordings.

The first two Specific Aims expand our testing of the hypothesis that the cerebellum is the site of an inverse dynamics model of the arm. Specific Aim 1 tests the hypothesis that the cerebellar nuclei are the output stage of an inverse dynamics model by examining the firing of interpositus and dentate neurons during a circular tracking task in which viscous and elastic force fields at varying magnitudes are applied to the hand. If the discharge of these nuclear neurons is modulated with the external force loads and encodes joint torques and/or upper limb EMG activity, then the discharge of cerebellar nuclear neurons is consistent with the output of an inverse dynamics model of the limb. Specific Aim 2 examines whether Purkinje cell discharge is consistent with the output of an inverse dynamics model when force control or force feedback is used to perform the task. Typically, tracking tasks require controlling the kinematics of the hand in relation to the movement of the target/cursor on the screen, and do not necessarily directly control force. It is possible that the failure to observe Purkinje cell modulation in relation to the external force fields is because the task did not depend on force control or force feedback. Specific Aim 2 proposes two additional circular tracking tasks. The first is an isometric task in which the monkey will track a visible moving target by applying forces to the force transducer handle. For isometric tracking force control is used to move the cursor and visual feedback is used to monitor performance. The second, "haptic tracking," requires the animal to track a haptic target consisting of a force detent in the shape of a bowl moving in the same circular path without vision. Haptic tracking uses force feedback to maintain the hand within the target. Purkinje cells will be recorded during the isometric, haptic and normal kinematic tracking as viscous and elastic loads are imposed. The analysis will determine if the simple spike modulation depends specifically on the controlled variable or the feedback.

Specific Aims 3-5 test the hypothesis that Purkinje cell simple spike discharge is the output of a forward internal model of the arm that predicts the upcoming arm kinematics. These Specific Aims examine whether the discharge of Purkinje cells is consistent with the properties of a forward internal model of the arm.

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 application is the continuation of our present NIH grant examining the nature of the movement signals in the cerebellum associated with visually-guided movements. These studies have shown how position, velocity and speed information are encoded in the discharge of cerebellar Purkinje cells and that manual tracking is composed of discrete subunits consisting of speed pulses highly correlated with the discharge of cerebellar Purkinje cells. Recent work has extended those findings, addressing how dynamic movement parameters (i.e., forces) are coded and the relationships to the kinematic parameters. We are investigating the processing of movement errors and how that error information is used to control movements both immediately and for long-term changes (i.e. motor learning).

The new application expands the previous work to test whether the cerebellum is the site of forward and inverse internal models of the arm. Our recent findings demonstrate that Purkinje cells in intermediate and neighboring lateral zones of lobules IV-VI are modulated in relation to hand position, direction of movement and speed during manual tracking. In contrast, these cells do not signal movement dynamics or muscle activity and therefore, cannot be the output of an inverse dynamics model of the arm. Instead, the simple spike discharge of these Purkinje cells signals arm kinematics, potentially consistent with the output of a forward internal model that predicts the state of the arm. Furthermore, the kinematic signals encoded in the simple spike discharge could be the requisite trajectory input to an inverse dynamics model located downstream of Purkinje cells. The new study will investigate these hypothesized models as described in 3A, above.

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.
-

These studies follow established methods for chronic recording single cells in the brains of behaving primates. Each animal is trained to perform controlled, visually guided arm and hand movements over a period of 6-12 months. 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 robotic manipulandum and track a moving target. Different types of target tracking (circular, random and random with perturbation) are required. 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 to the animal. 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 vacations 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 certain 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 task demands and the recording chamber has been implanted in the skull (6-12 months), data collection can be done. Neurons in the cerebellum are recorded as the animal performs circular and random tracking tasks. For these tasks, the monkey moves a robotic manipulandum to control a cursor displayed on a video screen placed in front of the animal. In some tracking tasks, the animal's hand will be displaced 3 cm by using the manipulandum. Electromyographic (EMG) activity is recorded using needle electrodes. Non-invasive systems such as video-based recording are used to monitor limb and/or eye movements.

The accepted standard for monkey studies of this type is to document the findings in several animals, each having a minimum of 100-150 usable cell recordings per recording site (i.e., chamber implant). Based on past experience, it

can take up to 4-5 months to collect the minimum number of cell recordings per recording site. However, recent advances in the design and development of multi-electrode probes have significantly reduced the time needed to collect the minimum number of usable cell recordings per site (2-3 months). The animal must then be re-trained to perform the task using the other hand.

At the end of the period of recording useful data, the head chamber is removed, all wounds allowed to heal and a second chamber is placed followed by additional cell recordings. 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 second surgery is considerably shorter. Most importantly, the second chamber placement 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. Performing additional survival surgeries is needed in order to meet the 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, determining recording locations. End point of study is when a sufficient number of cells have been recorded. Animals are maintained for 2-3 years.

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 five specific aims requires recording 100-150 neurons from 2-3 cerebellar hemispheres (i.e., 2-3 animals). Therefore, we are requesting a total of 9 animals.

The procedures are pain category B.

As described below in 4B the experimental design is based on the number of neurons and each neuron acts as its own control. The animals are not classified as control or experimental.

Generally, each monkey will participate in one study. However, the two studies associated with this protocol do have similar aims and use similar tasks that do not require the monkey to learn different behaviors across studies. Both studies require the animals to reach, grasp and move an object in each trial. From a training perspective, the tasks are the same. The similarity in aims is also such that the neural recordings for both studies are performed at the same chamber site and does not require the need for additional penetration sites.