

**ANIMAL USAGE FORM**

Version 2.51  
Updated 26 Aug 2004

AGENDA

FRM 06/13/06

IACUC Use Only			
IACUC Study #	0606A87286	Approved:	7/17/06
IACUC Chair:	Tom Molitor	RAR Veterinarian:	Cynthia S. Gillett

**Part A**

**0. Project Identification and Signatures**

**0A. Type of Application:**  New Protocol  3-year Renewal of IACUC #0307A50034  
(If this is a 3-year renewal, do not use language referring to the previous protocol or grant in this form.)

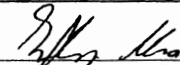
Anticipated Starting Date: \_\_\_\_\_

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

Temporal Strategies in Visual Cortex \_\_\_\_\_

**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>Ghose, Geoffrey M</b>	Phone Number: 612-625-8362
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U of M Employee ID: 3291772	Email: geoff@cmrr.umn.edu
U of M x.500 ID (ex. smith001): ghose002	University Department (if applicable): Neuroscience
Occupational Position: <input checked="" type="checkbox"/> Faculty <input type="checkbox"/> Staff (must be P & A) <i>Note: students cannot be principal investigator.</i>	
<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.	
 Original Signature of PI	Asst. Prof Title of PI
	6/21/06 Date

CIRCULATION COPY

*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: Geoffrey Ghose	Phone number: 5-8362	Email: geoff@cmrr.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).

Behavior depends on both the external environment and how subjects interpret that environment. One factor in that interpretation is past experience: subjects use strategies based on past experience to anticipate sensory events and plan appropriate motor actions. Preliminary findings suggest that sensory signals in visual cortex are modulated according to temporal strategies formed during training. The proposed experiments explore the effects of such strategies on the processing of visual information and the planning of visually guided behaviors. Animals will be trained in tasks with fixed timings to encourage the formation and use of specific temporal strategies. Simultaneous neural recording and behavioral measurements will reveal how these strategies affect behavioral performance and the neuronal signals underlying that performance. These studies have three specific aims. In the ~~first~~ specific aim, the effect of strategies on sensory processing will be studied in animals that have been trained in a motion detection task in which motion onset is determined by a consistent and periodic probability function. In the second specific aim, the effect of strategies on motor planning will be studied in animals that have been trained to produce regular eye movements in the absence of sensory cues. In the third specific aim, the nature and distribution of strategy related signals will be studied by comparing the responses of nearby cells during the performance of these tasks. Because these studies address internal task representations that have formed as a consequence of training, the experiments will provide valuable information on the neuronal representation of acquired knowledge. Additionally, by elucidating how strategies are used and implemented during normal behavior, these studies could provide important information for the development of treatments for a variety of learning and cognitive disorders.

#### 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.

Because these projects often require training animals for a year or more in extremely challenging tasks, the proposed project requires more than 3 years for completion. The first specific aim is largely complete, but the second specific aim has had more limited progress because one of the animals was untrainable and the other rejected all surgical implants. A third animal has been trained and data is now being collected, but the successful completion of all three specific aims were certainly require more time.

#### 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 I).
- 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.

We will perform chronic neurophysiological recording experiments from trained, behaving macaque monkeys. These experiments involve controlled fluid intake during behavioral training and neurophysiological recording, as well as recovery surgeries in which head-restraining posts, eye monitoring coils, and recording chambers are implanted. The animals are euthanized at the end of the experiment or sold or made available to other investigators requiring primates. The following chart indicates the experimental sequence for the animal.

**Magnetic Resonance Imaging of Cerebral Cortex:** MRI images afford a high resolution map of the animals' brains that can be used to guiding the placement of recording chambers and electrodes. These maps reduce the number of recording sessions for a given animal, and in some cases can obviate the need to euthanize the animal for histological verification of recording sites after we have finished our experiments. The procedure itself is noninvasive and can be considered diagnostic. Animals will be administered atropine (0.05 mg/kg), sedated with ketamine (15 mg/kg). A catheter will be inserted in a leg vein, and the animal will be intubated to maintain an airway. The animals will then be anesthetized with propofol (2 mg/kg IV), placed in a primate chair, and secured via a surgical implanted plastic headpost (see below). Anesthesia will be supplemented by continuous IV drip of propofol (0.2 mg/kg/min). Anesthesia level will be verified by continuous monitoring of pulse oxigenation, pulse rate, expired CO<sub>2</sub>, and respiration rate. The animal is wrapped in a chemical heating pad to maintain body temperature, which is monitored by a rectal thermister. After data acquisition, animals are returned to their cages and monitored until they are sitting upright.

**Behavioral Training:** Our experiments require that the animals direct their attention to specified positions in the visual field while holding their gaze on a fixed location. The duration of the training period depends on the task and the characteristics of the animal. It lasts up to 16 months, with the animal trained 5 days a week. Animals are first conditioned to get into and out of a primate chair and maintain a relaxed, normal posture while in the chair. The primate chairs are adjustable along several dimensions to allow comfortable sitting posture. Each animal is completely unrestrained within its home cage except during training or recording sessions which typically last about 4 hours. We use standard operant conditioning techniques with positive reinforcement (delivery of fruit juice or water for correct responses). Depending on the task, a correct response is either a hand (lever release) or eye movement made at an appropriate time. If the animal will use a lever, we normally train it to do the basic task without eye position requirements before implanting a scleral search coil and headpost (below). This typically takes 2-4 months. After the surgery, we wait at least two weeks before we begin to train under head restraint. The head post attaches to a mounting bracket on the primate chair and ensures the head stability that is needed for monitoring eye position and recording from individual neurons. The duration of head restraint is initially brief and is gradually increased during the process

of training. Animals adapt readily to head restraint. The head post is coupled directly to the skull and therefore generates no pressure on the skin. If the animal will make an eye movement for its response, the search coil and headpost are implanted before any task training. Once the animal is conditioned to head fixation and eye position can be monitored, it is trained to perform its task while holding its gaze on a small spot in the center of the video display. This fixation control allows us to know where the visual stimuli fall on the animal's retinas, and thereby create controlled, repeatable stimuli. With tasks involving eye movement responses, the animal is trained to move his eyes from the fixation spot to a specific target on the screen. Once the animal is proficient at the task, we begin to collect neurophysiological data.

**Neurophysiological Recording:** When training is completed, animals undergo a surgery in which a recording cylinder is implanted on the skull (details in section R8). When recording from surface regions on the cortex we use microelectrodes which penetrate the dura. For these recordings a small craniotomy (2-4 mm) is made inside the chamber under ketamine anesthesia the day before the start of recording. Delaying the craniotomy makes it possible to collect as much data as possible before the dura mater thickens to a point that makes microelectrode penetration impossible, which occurs over 3-6 weeks. In order to maximize data collection during this period there will typically be six recording sessions per week. When the dura becomes impenetrable, we either remove its overlying granulation tissue (under ketamine anesthesia), make a new craniotomy, or extend the existing craniotomy. For this procedure, which typically takes about 15 minutes, the animal is sedated with Ketamine (10 mg/kg IM). We typically make 3-4 craniotomies within each chamber and remove granulation 1 or 2 times from each craniotomy. When it is necessary to record from deep brain structures, a larger craniotomy is made at the time of surgery to allow maximal flexibility in electrode placement. For such recordings, a guide tube is used to bring the electrode to within 10 mm of the target. The guide tube is constructed from stainless steel hypodermic tubing and advanced through a grid within the recording chamber. The portion that punctures the dura mater and enters the brain is 28 gauge. When a guide tube is being used, both the electrode and the guide tube are soaked in disinfectant before insertion. Data are collected using metal microelectrodes that are advanced into the brain using a hydraulic microdrive. The microelectrodes are advanced transdurally in a procedure that does not require full aseptic methods or anesthesia. The brain itself does not have sensory endings, and the microelectrodes are smaller than fine hypodermic needles (~100 um O.D.). Electrodes are advanced until the action potentials from single neurons can be well isolated. On each day of recording the animal is put in its chair and brought to the animal prep area in the laboratory, where its head post is secured to a mating bracket. The external surfaces of the recording chamber cap and the surrounding bone cement are cleaned with a disinfecting solution (Novalsan), and the cap is removed by personnel wearing a surgical mask and gloves. Only sterile instruments and solutions enter the chamber. The interior of the chamber is rinsed with sterile saline and disinfectant is applied with sterile swabs. Secretions and debris in the chamber are removed by suction. A microdrive carrying a microelectrode that has soaked in Novalsan for 1 hour is fitted over the chamber, which is then filled with sterile mineral oil and sealed. The animal is placed in the recording room and the microelectrode is slowly advanced through the intact dura mater and into the brain. When the day's recording is completed, the microelectrode is retracted from the brain and dura mater. In the animal prep area the chamber is drained of oil and the microdrive is removed. The chamber is rinsed with sterile saline and disinfectant and a final sterile saline rinse, and closed with a sterilized cap. When all data have been collected from one recording cylinder, we typically implant another over a different region of cerebral cortex or the other cerebral hemisphere, and this is used for further recordings for about another 3 to 6 month period. Almost all animals received are implanted with 2 cylinders. It is less common for us to implant more than 2 cylinders, because there is generally no suitable scientific target or no room for access.

**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.

The second aim require recording 50-100 individual neurons from 2 to 3 animals. The third aim required recording from 10-40 pairs of neurons simultaneously from 2 to 3 animals. We are therefore requesting 6 animals in total.