

**PROTOCOL FOR ANIMAL USE AND CARE***Handwritten forms are not accepted***CNPRC**

EH&amp;S USE ONLY

**PROTOCOL # 10235****EXPIRES: \_\_\_\_\_**

Investigator		Contact	
Last Name:		Last Name:	
First:		First:	
Middle:		Middle:	
email:		email:	
Department:		Department:	
Phone / Fax:		Phone:	
After hrs. #:		After hrs. #:	

Species (common names):	Number:	Source:
Rhesus Monkey	27	CRPRC

<b>Project Title</b> Neurophysiology of Associative Memory in Aged Monkeys			
Overnight housing location::	CNPRC	Day use only :	
Animals will be maintained by:	<input checked="" type="checkbox"/> Vivarium <input type="checkbox"/> Investigator <i>(If investigator maintained, attach husbandry SOP's.)</i>		

**Procedures:** Provide a one or two sentence layman's description of the procedures employed on the animals in this project. This information will help the animal care staff understand any conditions they may encounter while caring for your animals.

Animals will be implanted with head posts, recording wells, and moveable microelectrodes for recording multiple single cells. MRI imaging is conducted longitudinally. Animals are trained to perform computer-controlled memory tasks for juice reward. Water control will be necessary for behavioral consistency, and the metabolic status will be monitored

**Special Husbandry Requirements:** Describe any special requirements your animals have with respect to **food, water, temperature, humidity, light cycles, caging type, bedding,** or any other conditions of husbandry.

The CNPRC animal care staff will not water the animals in the morning according to posted instructions. The investigator and the investigator's staff will water the monkeys in the laboratory during training sessions. The monkey will be supplemented with water upon return to the home cage by investigator per AUCAAC approved water restriction guidelines.

Other instructions for animal care staff: (check applicable entries)

Sick Animals	Dead Animals	Pest Control
<input checked="" type="checkbox"/> Call Investigator	<input checked="" type="checkbox"/> Call Investigator	<input checked="" type="checkbox"/> Call Investigator
<input checked="" type="checkbox"/> Clinician to treat	<input checked="" type="checkbox"/> Save for Investigator	<input checked="" type="checkbox"/> OK to use pesticides
<input type="checkbox"/> Terminate	<input type="checkbox"/> Bag for disposal	<input type="checkbox"/> No Pesticides in animal area
<input type="checkbox"/> Necropsy	<input checked="" type="checkbox"/> Necropsy	

**Hazardous Materials** *(only if in the animal room):*

Infectious Agents?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Radioisotopes?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Chemical Carcinogens?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Toxic Chemicals?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	

Funding source:	N. I. H. ( PHS )	Previously approved?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
Is the project already funded?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Previous protocol number (if any):	9350

**What Veterinarian or veterinary clinic will provide care for your animals? (check one)**

<input type="checkbox"/>	Lab Animal Health Clinic ( 2-0514 )	<input checked="" type="checkbox"/>	California Primate Research Center ( 2-0447 )
<input type="checkbox"/>	VMTH Large Animal Field Service ( 2-0292 )	<input type="checkbox"/>	Another Veterinarian

If you checked "Another Veterinarian", please provide:

Veterinarian:		Address:	
Day phone:			
Emergency phone:		Email:	

*If your veterinarian is not affiliated with one of the three service units listed above, please contact the campus veterinarian, 2-2357 (email pctillman@ucdavis.edu) for current information about training and record keeping requirements.*

**Summary of Procedures:**

**a)** Briefly describe the **overall intent** of the study. Include in your description a statement of your hypothesis, the objectives and significance of the study. Your target audience is a faculty member from a discipline unrelated to yours. Do not use jargon.

The goal of this project is to understand the neurophysiological basis of memory impairments which occur during the course of normal aging in primates. The hippocampal formation must be intact for long-term associative representations of visual objects to be formed in neural networks in the inferior temporal cortex. The specific hypothesis tested in the present proposal is that changes in single cell responses in inferotemporal cortex, that occur as a result of associative learning, will be altered in aged, memory-impaired primates. Specifically, altered synaptic plasticity of the backward projections from hippocampal formation structures to inferior temporal cortical areas should lead to reduced associative modifications in old neural networks. The extension of these ensemble recording methods to the aged non-human primate brain has the potential to lead to important direct insights into the mechanisms of memory impairment in aged humans, and into possible treatment strategies.

**b) Procedures employed in this project:**

Please check the appropriate boxes if any of these procedures will be employed in your project:

<input type="checkbox"/> Monoclonal Antibody Production **	<input checked="" type="checkbox"/> Food or water restriction	<input checked="" type="checkbox"/> Special diets; food or water treatment.
<input type="checkbox"/> Polyclonal Antibody Production **	<input checked="" type="checkbox"/> Non-recovery surgical procedures	<input type="checkbox"/> Induced illness, intoxication, or disease
<input type="checkbox"/> LD 50 or ID50 studies.	<input checked="" type="checkbox"/> Survival surgical procedures	<input type="checkbox"/> Death as an endpoint (see i below)
<input checked="" type="checkbox"/> catheters, blood collection, intubation	<input checked="" type="checkbox"/> Multiple survival surgery	<input type="checkbox"/> Trapping, banding or marking wild animals
<input type="checkbox"/> Prolonged restraint. (8 hrs+)	<input checked="" type="checkbox"/> Behavioral modification.	<input type="checkbox"/>
<input type="checkbox"/> Fasting prior to a procedure.	<input type="checkbox"/> Aversive conditioning.	<input type="checkbox"/>

**\*\* If this protocol only describes antibody production, you may use the attached antibody production page in lieu of completing section c below.**

c) Describe the use of animals in your project in detail, with special reference to any of procedures checked above. Include any physical, chemical or biological agents that may be administered. List each study group, and describe all the specific procedures that will be performed on each animal in each study group. Use terminology that will be understood by individuals outside your field of expertise. (Note: This cell will expand to whatever length you require. You may make this section as long as you wish, but try to be concise. Some projects may require one or two pages.)

### General strategy

The general strategy of these experiments is to first acclimate the monkeys to the testing room and apparatus. They are then trained to perform behavioral tasks that demand their memory and attention (**B-1**). Once the monkeys master the memory tasks, their eye movements will be monitored while they perform the tasks (**B-2**). This requires that they be implanted with a headpost for head restraint and their eye movements will be monitored via either a search coil or infrared eye tracking system (**S-1**). Once the monkeys are trained on the behavioral tasks, neural activity is recorded from temporal lobe brain structures (**C-1** through **C-3**). During the course of this experiment, we will be involved in the ongoing process of improving the electrophysiological recording methods. This means that, from time to time, we will need to test electrodes and electrode manipulators in anesthetized animals (**A-1**). Animals will be anesthetized for the acute experiments (**A-1**) and for surgical procedures (**S-1** through **S-2**) as described below, but for behavioral training (**B**) and chronic electrophysiological recording (**C**), the animals will be sitting in their chairs with head restrained by a head post. In order to create accurate neuroanatomical maps for each monkey, MRI imaging will be obtained from each monkey (**I-1**). This allows accurate stereotaxic placement of the recording electrodes. During the recording experiments, the trajectory of the electrodes will also be assessed using X-ray and CT scan methods (**I-2**). Two additional collaborative experof the young monkeys will range between 8 and 13 years (mature, young) and monkeys of 25 years or greater (old, equivalent to 75+ human years). The total number of survival neurosurgical procedures conducted on each monkey in the primary experiment is 4 (if everything goes as planned): a) the headpost is first attached at the back of the skull to provide head restraint in the primate chair (if eye coils are implanted they are done at this time); b) attachment of the recording wells, one on each hemisphere of the young monkeys will range between 8 and 13 years (mature, young) and monkeys of 25 years or greater (old, equivalent to 75+ human years). The total number of survival neurosurgical procedures conducted on each monkey in the primary experiment is 4 (if everything goes as planned): a) the headpost is first attached at the back of the skull to provide head restraint in the primate chair (if eye coils are implanted they are done at this time); b) attachment of the recording wells, one on each hemisphere, through which the electrode movement device is eventually attached (after craniotomies); c) and finally craniotomies to remove the skull from inside the recording well, first on one hemisphere; d) then at a later time a craniotomy is performed inside the recording well on the other hemisphere. In addition the males will have vasectomies to allow opposite-sex paired housing for these monkeys. Thus, the total number of survival procedures for the main experiment (with the caveat that we are developing the procedures as we go, and may need to modify them, as necessary) typically will be 4 for the females, and 5 for the males. The anticipated timeline for the primary experiment (again, this work has not been done before, and thus, the timing is estimated) will be for 2 to 3 young/old monkey pairs to be in behavioral experiments throughout the 5 years, and 1 young/old monkey pair will be implanted with electrodes to allow simultaneous electrophysiological recordings with behavioral testing, continuously throughout this time. We will continue with these experiments until 6 young and 6 old monkeys (6 young/old monkey pairs) have been thoroughly behaviorally tested and chronic electrophysiological

recordings have been made from the medial temporal lobes of both hemispheres of 12 animals. We will also take longitudinal MRI images on the 12 primary monkeys in the experiment, throughout the years of the experiment. Other monkeys will be used to develop behavioral procedures, procedures to improve MRI imaging methods or to test novel approaches that might enhance the yield in our electrophysiological experiments.

#### **Acute experiments (A-1)**

Experiments will be performed on anesthetized Rhesus monkeys to collect electrophysiological data from medial temporal lobe structures. Anesthesia (Isoflurane and Fentanyl) is maintained (including i.v. lines for fluids) for a period ranging from 4-10 hrs by the CNPRC veterinary staff. Direct measurements of neuronal activity will be made from the brain using microelectrodes held by stereotaxic arms, and advanced through a craniotomy. Throughout the procedure the animal is mounted in the stereotaxic apparatus on a table and actively ventilated and monitored. At the end of the experiment the animal is euthanized with an intravenous overdose of barbiturate, perfused transcardially with fixative and the brain is removed for histology.

#### **Behavioral training (B-1)**

Before entering into the behavioral (Section B) and then semi-chronic electrophysiological (Section C) components of the experiment, the monkeys are assessed by veterinary and technical staff for their suitability, both psychologically and physiologically, to participate in experiments motivated by water reward. These include a specific tests of the effects of water restriction on individual animals while under careful physiological monitoring conditions (typically for a period of 2 weeks, blood, urine, etc.), screening out animals with repeated psychological symptoms of depression or other conditions noted in their records or by staff at the Center that may not be conducive to the kinds of behaviors required in these experiments. Once the Center veterinarian and technical staff have passed a given monkey on this initial screening, the monkey will be entered into the main project, and will be fitted with a collar so that they can be chair-trained (see below). In the first stage of the experiment, monkeys will be trained to perform a behavioral task for fluid reward, that does not require eye fixation, or head restraint. Each animal is initially acclimated to a primate chair by CNPRC animal care staff, or investigators who have been trained in chairing methods, which has been carefully designed for the animals comfort. After the acclimation period, they readily climb into the chair, using the pole and collar technique. In this phase of training, animals will be restrained only at the neck and are free to turn about freely. They will also be taught to hold a bar in the front of the chair. The task involves viewing visual stimuli on a computer screen and releasing or holding a bar for specific stimuli. Monkeys are presented with fluid reward for responding appropriately. The training session lasts from 2-6 hours per day, typically 5 days per week for several months. During critical periods, the animals may be tested up to 7 days per week. The animals water intake will be regulated according to the AUCAAC approved water restriction guidelines (for details on water control see below **W-1**).

#### **Behavioral training (B-2)**

Eye movement monitoring is a critical control in these experiments. It is important to know that the monkeys are looking at the stimuli and attending to the task for accurate behavioral analysis. The animal may make errors on the task because it is looking away from the monitor. We need to separate

these types of errors from errors in which the animals misidentify stimuli. If the animal is looking at the center of the screen throughout the trial then we can infer that errors are not due to attention failure. More importantly, direction of gaze influences the firing properties of neurons in visual brain regions.

After initial training (B-1), the monkeys will have a head post attached at the back of the skull to provide an anchor to restrain its head in order to monitor its eye movements during the task. The animal will be given approximately two weeks to recover after this surgery before the head post is attached to the chair. The standard method for monitoring eye movements in primates during physiological experiments is to implant an eye coil which is attached to a small connector on the head post (for surgery details see S-1). The eye coil is a loop of fine insulated wire that acts like an antenna inside a magnetic field. Then the animals are placed inside a magnetic field, and eye location can be calculated based on the location of the wire coil around the monkeys eye.

We have adapted new technology, the infrared eye tracking technique, to our experiments, and hope to use this method exclusively for eye tracking. In this case, the monkeys still need to have their heads restrained, but since eye movement is determined by an infrared camera, there will be no need for the eye coil, thus eliminating short-term discomfort (2-3 days) experienced by the monkey after the eye coil is implanted. We will only conduct the eye coil procedures if we find that experimental accuracy is compromised by the use of the infrared system. In the tests we have made so far, we believe that we will be able to use the infrared system for most applications.

The general types of behavioral testing conducted with head restraint involve viewing a computer monitor, fixating appropriately, responding to visual stimuli on the screen by holding or releasing a bar, and receiving fluid reward for correct responses.

### **Behavioral training (B-3)**

In order to compare our behavioral results in the computerized tasks to other learning and memory studies that have compared performance of young and aged monkeys, we would like to further test our monkeys in the Wisconsin General Testing Apparatus (WGTA). This testing is conducted in a large cage (4x3x3 ft). The experimenter sits in front of the cage and rewards correct choices with preferred food items by using a series of screens in the cage that allow visual observation but no access to the objects, or visual and tactile access to objects. No water or food restriction is required with this type of training because the number of trials in a given day is fairly small (usually around 30 depending on the specific task). The animal is allowed to move freely in the testing cage during testing. The basic requirement of these tasks is for the monkey to remember an object that it has been shown over several different delay intervals, or to choose a novel object over a relatively familiar object after delays of varying intervals. The experimenter systematically presents "junk objects" to the monkey, and, when the task demands are met, the monkey is allowed to retrieve the preferred food item which resides in a well underneath the correct object. The maximum amount of time the animal would spend in testing is 4 hours. An experimenter is present with the animal at all times.

### **Head post surgery for eye movement monitoring (S-1)**

In this surgery, a head post will be attached to the skull, and eye coils (if used) will be placed. Animals will be pretreated with Atropine and

lightly anesthetized with Ketamine, then anesthetized with isoflurane as a general anesthetic. Anesthesia and surgery preparation will be administered by the veterinary staff. During surgery, fluids will be administered I.V., and heart rate, temperature, blood pressure, and respiration will be monitored. During the surgery, the scalp and underlying muscles will be retracted. Titanium orthopedic strips will be fastened to the posterior part of the skull using titanium screws, and extra screws (8-10) will be fixed in the skull to anchor the head post. The holes for the screws in the skull are started using a tapping tool that is rotated by hand. Dental acrylic will cover the titanium strips and screws, and the titanium headpost will protrude outside the acrylic far enough so that it will be able to stabilize the head in the primate chair when the monkey is attached to the head restraint bar. The wound margins will be sutured if necessary, and cleaned thoroughly. Analgesics will be prescribed by the veterinary staff as necessary [in the past after head post mounting, 2 days oxymorphone (0.15 ml/kg) 3 times daily i.m. injection; 3 days Buprenex (0.03 mg/kg), and antibiotics (in the past 5 days Sefazolin)]. The head post surgery must be done before the animals can begin behavioral training on the computerized tasks that require precise fixation.

If a search coil is implanted, the conjunctiva is opened and a coil is placed around the globe, then the tails from the wire are threaded underneath the skin to a point where they can be attached to a connector on the monkeys implants.

Although young monkeys only experience minor inflammation and discomfort for a few days following eye coil surgery, it is our expectation that the aged monkeys in our experiment may have more difficulty recovering from the eye coil surgery. Furthermore if the eye coil wire should break during a critical period in training or recording period, then the animal must return to surgery for replacement or repair. These are the primary reasons that we will use infrared eye tracking cameras to monitor eye movements as much as possible. In the event that this is not possible, we would fall back on the traditional eye movement monitoring techniques.

#### **Chamber attachment surgery, craniotomy (S-2)**

This surgery involves the same preparation as above in S-1, except that recording chambers will be fixed to the skull. The chambers are standard Cryst plastic recording wells, and are held in place by 5 to 7 titanium screws placed around the periphery of the chamber, and are covered with dental acrylic. This procedure can be done one of two ways: perform a craniotomy and attach the chamber over the craniotomy; or just attach the chamber to the skull, and at a later time perform the craniotomy inside the well. The procedure that we have used to date is to wait to do the craniotomy until we are ready to attach the hyperdrive apparatus, but we are still optimizing all these procedures and need the flexibility to do it either way, as circumstances dictate. Again, animals will be pretreated with Atropine and lightly anesthetized with Ketamine, then anesthetized with isoflurane as a general anesthetic. Titanium orthopedic screws will be used as an anchor to the skull (with the screw holes in the bone hand-started as described in S-1) and dental acrylic will be used to fasten the chambers to the screws and skull. Analgesics will be prescribed by the veterinary staff as necessary, and antibiotics continued over a prescribed course. If the craniotomy is performed after the recording chamber attachment, then the monkey will be anesthetized and treated exactly as above for this surgery. For the craniotomy the skull is drilled just smaller than the recording well with a dental drill. Every effort is made to remove the bone flap in a

manner that minimizes trauma to the dura.

### **Chronic Experiments (C)**

Once the animal has reached a criterion level of behavioral performance in the computerized tasks, if a craniotomy has not already been performed (S-2), it is performed at this time. The dura matter is left intact, and the chamber is sealed with a cap. Electrophysiological recordings of neuronal activity from the temporal lobe is conducted daily on alert Rhesus monkeys performing visual fixation and computerized visual memory tasks. Recording wells are affixed over both hemispheres so that we can perform craniotomies confined to the wells on each hemisphere and can record from medial temporal lobe structures in both hemispheres. Only one hemisphere will be recorded at a time, primarily because of limitations of amplification and computer acquisition equipment. We currently can record simultaneously from 52 channels, and dual hemisphere recording would require a system capable of simultaneously recording 100 channels. If such a system becomes available to the project, then it would be possible to record from both sides of the brain simultaneously. During the recording sessions the monkey sits comfortably in a standard primate chair with head restraint using the headpost. As in training, the animals' water intake will be controlled to optimize behavioral performance.

#### **Electrophysiological recording: Acute electrode recording (C-1)**

Like all of the experiments in this category (C), the monkeys will be sitting in a chair performing a visual memory task, and they will have a headpost (S-1) a recording chamber attached, and a craniotomy (S-2). We refer to this specific type of recording session as acute recording, because the electrodes are placed and removed on a daily basis, even though the chamber and head post are chronically implanted. Electrodes will be advanced into the brain through the dura matter with mechanical micromanipulators. The cap of the recording well is removed and the well is rinsed with a dilute disinfectant solution. The micromanipulator is then placed on the well and fixed in place. The electrodes will be advanced into the brain where they will remain for the duration of each daily session. The animals quickly become habituated to the microdrive and electrode placement.

#### **Electrophysiological recording: Semi-chronic electrode recording (C-2)**

In order to document the changes in brain activity involved in learning, it is necessary to record the activity of the same brain cells over multiple days. This can be accomplished by using indwelling electrodes that can be left in the brain for several days. In addition, recording of neuronal activity from several microelectrodes typically requires a setup time of anywhere from 15 minutes to 1 hour depending on the number of electrodes introduced. This relatively long setup time is not optimal because it limits the amount of time available to collect experimental data in a single session. To improve upon this method an additional technique will be employed to chronically implant several microelectrodes by attaching a microelectrode manipulator onto the chamber, and leaving this apparatus in the brain for a month or more. We have successfully tested this method for a 30-day period, and are still recording successfully from this animal.

Before beginning the combined behavior/electrophysiological recording component of the experiment, animals will be pretreated with Deprakote 1 day before, the day that cannulae are inserted into the brain, and one day after, to reduce the risk of mechanically-elicited cell excitation. The day of the hyperdrive attachment procedure, animals are given antibiotics and

atropine and lightly anesthetized with Ketamine then anesthetized with isoflurane as a general anesthetic. Anesthesia and surgery preparation will be administered by the veterinary staff. During surgery, fluids will be administered I.V., and heart rate, temperature, blood pressure, and respiration will be monitored. They will be placed into a stereotaxic instrument to help stabilize the head. The inside of the chamber will be washed thoroughly with a disinfectant solution prior to the insertion of the recording assembly. The insert and associated manipulators and electrodes will be sterilized prior to implantation. The micromanipulator insert ("the hyperdrive device") containing the microdrives, guide tubes and electrodes, will be placed inside the recording chamber and fixed in place using set screws. The guide cannulae will be lowered through the dura at this time. Because the mechanical stimulation of lowering the guide cannulae might cause excitatory electrical activity that could lead to seizures, the veterinary staff will give i.v. Valium right before the cannulae are lowered into the brain. This hyperdrive attachment procedure takes about an hour. After the monkey is released by the veterinary staff to participate in the experiment, the electrodes will be advanced, by turning one of the 14 screws on the hyperdrive apparatus. The brain has no pain receptors, so this can be done while the monkey is sitting with its head restrained in the primate chair. We can turn in 10-20 micron increments until good recordings of neuronal activity is obtained on each channel. We will simultaneously monitor EEG to ensure that no abnormal electrical activity is caused by these small movements. At the end of the recording/behavior session the chamber will be flushed (every day) alternating daily with betadine and novasan, if the system is open, and an encasement cap will be fit snugly over the assembly to seal it. This is a tight, press fit over O-rings, and set screws maintain the seal of the cap so that the monkey cannot remove it. We have just performed a hyperdrive attachment on another monkey in which the drive was completely sealed around the cannulae and the well. The seal was made with sterile silastic. This attachment has allowed recording from multiple single cells over a 30 day period, and may be the preferred procedure for future implants. On subsequent days the recordings will be initiated by simply removing the encasement cap, attaching the headstage amplifier to the hyperdrive connectors that make contact with the electrodes, and making any adjustments to the electrode depth to optimize the isolation of cells.

After several weeks of recording, when the electrodes have been extended to their full depths, the monkey will be given a prophylactic dose of Depakote, immobilized with Ketamine (under supervision of the veterinary staff) the electrodes will be withdrawn, the hyperdrive assembly and encasement attachment removed, the chamber flushed with the disinfectant solution, and the disinfected recording well cap replaced. This should take 15 to 20 minutes. Careful evaluation will be made of the animal before and after each behavior/electrophysiological recording session to watch for signs of infection. Neuronal activity is a sensitive indicator of brain function and will be utilized as an additional means to evaluate the health of the animal. If at anytime during the procedure the animal shows any sign of infection, appropriate antibiotic treatment will be administered. If necessary the electrodes will be retracted, the insert removed, the chamber flushed and disinfected and the cap replaced. When the hyperdrive device is off, the chamber is still cleaned at least 5 days per week.

#### **Electrophysiological recording: Chronic electrode recording (C-3)**

Depending on the quality of data recorded with the above procedures, we may turn to the procedure with which we have years of experience in the rat, of

chronically implanting multiple microelectrodes in the brain. Our aim is to record over a period of 3-4 months neuronal activity from 1-20 microelectrodes in temporal lobe brain structures. To achieve this we will perform a simple sterile surgical procedure that follows the two previous surgical procedures conducted on each animal to implant the eye coils, if necessary, and the headpost and recording chambers. The animal will be anesthetized with a Ketamine (10-15 mg/kg) and maintained under isofluorane with fluids maintained and monitored by veterinary staff. The monkey will be mounted in a stereotaxic apparatus, and then, using sterile procedures, the cover of the recording chamber will be removed and the chamber rinsed thoroughly with a disinfectant such as betadine followed by sterile saline. A small craniotomy will be made in the bone (using a small dental drill, combined with water drip and suction) overlying the cortex to be studied (approximately 5 mm in diameter) and the dura mater opened. The size of the opening in the dura will be as small as possible to allow for the placement of the recording electrodes. The electrode assembly (previously sterilized) will be positioned over the craniotomy and the tips of the guide tubes containing the microelectrodes inserted inside the dura to rest just above the surface of the cortex. The opening will be sealed with sterile gel foam and biocompatible Silastic, rinsed with an antibiotic solution, and then covered with dental acrylic to insure a tight seal. The associated micromanipulator will be fixed in place inside the recording chamber using dental acrylic. The monkey will be given appropriate post-operative analgesics under veterinary supervision. Two to five days following this procedure the animal will resume its normal behavioral training. Recordings will be obtained by slowly advancing each microelectrode into the cortex. The assembly will be inspected on a daily basis during each recording session and can be cleaned if necessary using a disinfectant solution. Once the recordings are completed the assembly will be removed. A new small craniotomy would then be made and the electrode assembly implanted at a different site within the same recording chamber. This procedure could potentially be used to target multiple sites within each chamber at 1-3 month intervals.

This protocol may have several advantages over the procedures used in C-1 and C-2. It does not require a large craniotomy. Therefore the chances of infection are reduced. The technique also does not require repeated thinning of the dura mater and removal of re-grown bone. This will reduce the number of procedures that must be performed under anesthesia. The disadvantage of this method is that each electrode array is cemented to the skull and thus it can sample only a limited number of sites within the temporal lobe. While this technique may eventually be found to be the optimal recording technique, it has not been thoroughly tested in primates, as has the semi-chronic procedure.

#### **Implant maintenance (M-1)**

Careful attention will be paid to the head implant to reduce the possibility of infection. There are two main components involved in implant maintenance: 1) maintaining the wound margin, and 2) maintaining the craniotomy by flushing and dural scraping. The frequency of cleaning depends on the state of the implant and the individual monkeys physiological response to the implanted hardware. Some monkeys do not mind gentle cleaning and hair cuts for wound margin maintenance, while sitting in their chairs with their heads restrained. If a monkey is clearly disturbed by cleaning or needs more vigorous cleaning, it may have to be lightly anesthetized with Ketamine for treatment. Chambers with open craniotomies will be cleaned regularly (3 to 5 days per week) by flushing the chamber with biocompatible disinfectants

while the monkey is head restrained, and, in our experience, they adapt to this procedure quickly.

Due to dural growth it is necessary every several weeks or months to thin the dura matter and remove excess bone from the craniotomy during the acute implant procedures, or in between implants in the semi-chronic procedure. The animals will be given a pre-op dose of antibiotics and then anesthetized with an I.M. injection of Ketamine (15 mg/kg). The cap of the recording chamber is removed, the chamber flushed with the disinfecting solution. Using sterile techniques, the layer of connective tissue overlying the dura is removed using forceps and a sharp periosteal elevator. The dura mater is thinned by removing the outer layers using forceps and a curved needle or drilled away using an electrical dental drill or Dremel tool. Any excess bone that has grown back around the margins of the cranial opening is removed. At the end of the procedure the chamber is disinfected by rinsing with the disinfecting solution. The cap is replaced and the animal recovered.

#### **Imaging Procedures (I-1))**

There are several reasons why MRI imaging will be conducted on our monkeys. The first is to allow for precise anatomical measurement and placement of electrodes that will be used during physiological recording. The second is to obtain volumetric measurements of specific brain areas of interest for correlation with our behavioral and electrophysiological data on the same animals. The third is to obtain resting metabolic measurements using a contrast enhancing agent. The MRI imaging will all be conducted at the Sacramento Medical Imaging Center. Animals will be transported to Sacramento in a Primate Center van. One SRA, one veterinarian, and an investigator from the project will typically be present at all procedures. Before the scan, the primate is anesthetized with Ketamine (15mg/kg) and Medetomidine (20-40 mcg/kg). When fully anesthetized, the monkey is placed in an MRI-compatible stereotaxic frame and placed inside the magnet. The animals will be monitored including heart rate, respiration and oxygen saturation. For the resting functional metabolic scans (that assesses cerebral blood volume), an image contrast-enhancing agent Gadodiamide (gadolinium, Omniscan) will be administered to improve visualization of small structures. The dose will be 0.1-0.3 mmol/kg, injected intravenously, and veterinary staff will be there to monitor the monkey. We expect no adverse effects as a result of use of this compound, which is used widely in human MRI studies; however, veterinary staff can administer antihistamines or steroids if allergic reactions should occur. Gadolinium compounds are extremely safe and are readily eliminated by the kidneys. Rare side effects of use in humans are nausea and hives. In addition to MRI imaging, after the procedure to insert the hyperdrive apparatus and microcannulae, the monkeys will be given X-ray scans to allow assessment of the trajectory of the cannulae through which the microelectrodes will be lowered, and CT scans during and at the end of recording when the electrodes are fully extended. The CT and MRI scans will be co-registered to estimate electrode location and will be used to compare with the final histological analysis.

#### **Water Restriction (R-1)**

This experimental protocol requires that monkeys perform brief (2-3 sec) visual fixations of a small target, accompanied by behavioral discrimination of a visual stimulus, one to five thousand times a day, at least five (and sometimes 7) days per week. These sessions typically range in duration from 2-6 hours.

Fluid rewards will be preferable to food rewards because of 1) the number of trials necessary to collect physiological data and 2) drinking causes less electrical artifact during recording sessions than does chewing. Maximizing daily performance minimizes the number of days that the animal will be engaged in electrophysiological recording sessions. To insure a stable level of behavioral performance it is necessary to control the animals' water intake carefully since preferred fluids will be the primary reward for performing the task. During training, each animal will be allowed to work for as much fluid as it desires during each session. Typically, animals will be given extra food treats during work breaks. When animals are satiated, they will typically stop working and sometimes fall asleep.

To insure that the animals remain in good health each day, the following will be checked: behavioral activity, general appearance, skin turgor, appetite, feces, urine and weight. Animals will be weighed at least weekly while under water control. The animals are required to perform attention-demanding tasks, and health problems can also be detected if behavioral deterioration occurs. Signs of behavioral change will be continually monitored. All water restriction will be conducted in accordance with AUCAAC approved guidelines.

#### **Experimental Control of Environmental Enrichment (E-1)**

We feel that environmental enrichment is extremely important for normal brain function in macaques and is therefore critical for our experiments. Therefore we would like to have more environmental enrichment, above and beyond that which is required by CNPRC SOPs. For this reason, we would like to assume responsibility for all environmental enrichment given to our animals.

Food enrichment. In these experiments, lack of control over food enrichment with high fluid content or high salt content, may influence the monkeys health and performance on a day-to-day basis. In the past year, we have given our monkeys additional food enrichment (i.e., vegetables, fruits, dried fruits, nuts) consistently at least 6 days per week. This is documented in the running water log kept in our animal colony room. We would like to continue this procedure in a consistent manner (i.e., at least 6 days per week) by our project staff, rather than the 2 days per week the monkeys are currently given additional food supplements by the CNPRC Enrichment Staff. We will do this in consultation with the Enrichment Staff.

Manual manipulation. We have purchased a number of colorful plastic, rubber, or soft toys from pet stores for the monkeys in our project under the guidance of the Enrichment Staff. Most of these toys are filled with dried fruit or nuts prior to presenting them to the monkeys in the holding area.

Visual stimulation. Because our experiments involve visual learning, and experience can directly influence the outcome of our results, we would like to control when the animals are given TV or video visual stimulation. We have a TV and VCR in the animal holding area in SS2021, and the animals are allowed to watch TV and/or videos while in the holding area prior to and after being tested each day. We will also consult with the Enrichment Staff to find appropriate videos. This way, our animals get consistent visual stimulation each day, rather than sporadically as currently provided by Staff. All animals will be monitored carefully for behavioral abnormalities indicating poor adaptation to testing. These research protocols are heavily dependent on behavioral measures and requires that monkeys be unstressed, healthy, and cooperative. As stated above, all water restriction

will be conducted in accordance with AUCAAC approved water restriction guidelines.

**Social contact.** Perhaps the single most important variable for monkey psychological well-being is close contact with other conspecifics. The potential danger of pair housing animals is physical harm to one or both monkeys if the monkeys are socially incompatible. In our project, we would like to pair house all monkeys. It is the recommendation of the Enrichment Staff that opposite sex pairings are more likely to be successful than same sex pairings, especially in the case of the aged individuals. Since pregnancies resulting from opposite pairings would seriously compromise the goals of the research, all male monkeys in this project will need to be vasectomied, so they can be paired with age-matched female monkeys. The vasectomies will be conducted by CNPRC veterinary staff. We have been successful in housing two pairs of monkeys together (1 young female, 1 young male and 1 old female, 1 old male).

**d) Study Groups and Numbers:** Define, in the form of a table, the numbers of animals to be used in each experimental group described above. The table may be presented on a separate page as an attachment to this protocol if you prefer. The Normal format should be three columns: Study Group, Procedure, Number of animals. The number of rows should follow from the number of study groups; **you may add as many rows as you require.** The chart must fully account for the number of animals you intend to use under this protocol. Assign each group to an invasiveness category according to the chart below.

Group	Procedures / Drugs	Number of Animals	Category
A-1	Craniotomy	3/yr	3
B-1	Chair restraint, behavioral training, water restriction	6/yr	1
B-2	Head and chair restraint, eye movement monitoring, behavioral training, water restriction	6/yr	2
B-3	Behavioral testing in the Wisconsin General Testing Apparatus	6/yr	1
C-1	Electrophysiological recording, Head and chair restraint, eye movement monitoring, behavioral training, water restriction	6/yr	2
C-2	Electrophysiological recording, Head and chair restraint, eye movement monitoring, behavioral training, water restriction	6/yr	2
C-3	Electrophysiological recording, Head and chair restraint, eye movement monitoring, behavioral training, water restriction	6/yr	2
I-1	MRI, X-ray and CT imaging	8-12/yr	2
S-1	Head post surgery	6/yr	3
S-2	Chamber implant surgery, craniotomy	6/yr	3

M-3	Implant maintenance, dural cleaning	6/yr	2
	<i>Note: with the exception of A-1, this list of procedures refers to a series of procedures, rather than separate study groups so the total number of animals used in a year will not exceed nine.</i>		

#### Categories of invasiveness

Category	Description
1	Little or no discomfort or stress <b>Examples:</b> domestic flocks or herds being maintained in simulated or actual commercial production management systems; the short-term and skillful restraint of animals for purposes of observation or physical examination; blood sampling; injection of material in amounts that will not cause adverse reactions by the following routes: intravenous, subcutaneous, intramuscular, intraperitoneal, or oral.
2	Minor stress or pain of short duration <b>Examples:</b> cannulation or catheterization of blood vessels or body cavities under anesthesia; minor surgical procedures under anesthesia, such as biopsies or laparoscopy; short periods of restraint beyond that required for simple observation or examination, but consistent with minimal distress
3	Moderate to severe distress <b>Examples:</b> major surgical procedures conducted under general anesthesia, with subsequent recovery; prolonged (several hours or more) periods of physical restraint; induction of behavioral stresses such as maternal deprivation
4	Severe pain near, at or above the pain tolerance threshold <b>Examples:</b> exposure to noxious stimuli or agents whose effects are unknown; exposure to drugs, chemicals, or infectious agents at levels that markedly impair physiological systems and which cause death, severe pain, or extreme distress; Surgical experiments which have a high degree of invasiveness.

Further descriptions of these categories are included in the instructions following this document.

e) **Rationale for species and numbers:** How did you determine that 1) the species choice was appropriate and 2) the number of animals in each study groups was the minimum number necessary to achieve sound scientific results?

1. Rhesus monkeys are capable of learning complex memory tasks, comparable to mature and aged humans. They are also capable of carrying out the behavioral protocol required for behavioral neurophysiology. It is also the species of choice because young monkeys have already been electrophysiologically examined in the memory tasks that will be used for the age comparison. A vast body of research on primate visual memory has been carried out using rhesus macaques. Therefore, we will be able to interpret our experiment in the context of existing findings on memory in primates with the possibility of relating these findings to memory function in humans.

2. It is generally assumed in the scientific literature that at least 3 subjects/experimental group are necessary to have the statistical power to detect robust differences between groups. In our experiment, we will be testing a total of 12 animals. Of those 12 animals, 6 will be aged monkeys and 6 will be young controls, and this will be conducted over several years. We will need up to 3 animals for our acute experiment (A-1) each year, and replacements for any of the 6 young or 6 old animals that might become ill or die before the end of the experimental procedures. Inter-individual variability calls into question the results obtained from fewer subjects.

f) **Surgery:** If the project involves survival surgery, where will the surgery be conducted?

Building:

CNPRC

Room:

Surgery room

Who will be the surgeon?

g) **Anesthetics, Analgesics, Tranquilizers, Neuromuscular blocking agents:**

Post procedural analgesics should be given whenever there is possibility of pain or discomfort that is more than slight or momentary. If postoperative analgesics are not to be given, justify the practice under part (i) below.

Provide the following information about any of these drugs that you intend to use in this project.

Species	Drug	Dose (mg/kg)	Route	When and how often will it be given?
Rhesus	Ketamine	10-15mg/kg	im	
Rhesus	Medatomadine	20-40 mcg/kg	im	
Rhesus	Atipamizole	0.15mg/kg	im	
Rhesus	Atropine	.04 mg/kg	im	
Rhesus	Telazol	5-8 mg/kg	im	
Rhesus	Oxymorphone	15 mg/kg	i.m.	3 times/day, 3 days postop
Rhesus	Valium	0.5-1 mg/kg	iv	
Rhesus	Fentanyl	7-10 mcg/kg/hr	iv	
Rhesus	Isoflurane	1-2%	inhalation	1-5 hours
Rhesus	Buprenex	0.01-.03 mg/kg	im	3-10 days, pre and post op
Rhesus	Depakote	250mg	oral	for 3 days

**h) Neuromuscular blocking agents** can conceal inadequate anesthesia and therefore require special justification. If you are using a neuromuscular blocking agent, please complete the following:

Why do you need to use a neuromuscular blocking agent?

N/A

What physiologic parameters are monitored during the procedure to assess adequacy of anesthesia?

The acute experiments are preceded by a surgical procedure which typically lasts several hours. During this time the animal is anesthetized but not paralyzed. This allows the experimenter to determine and insure that an adequate level of anesthesia is obtained during the surgery. The level of anesthesia sufficient for surgery is then maintained throughout the entire experiment. This procedure ensures that the animals do not suffer from pain or discomfort during the experiment when it becomes difficult to assess the level of anesthesia. Following the surgery, the remainder of the experiment is devoted to electrophysiological data collection. The condition of the animal is continuously monitored using electrocardiogram, heart rate, expiratory CO<sub>2</sub> concentration, and rectal body temperature by the veterinary staff at the CNPRC.

Under what circumstances will incremental doses of anesthetics-analgesics be administered?

Additional anesthesia is administered if any manipulations of the animal cause an increase in heart rate.

**i) Adverse effects:**

Describe any potential adverse effects of the experiment on the animals (such as pain, discomfort; reduced growth, fever, anemia, neurological deficits; behavioral abnormalities or other clinical symptoms of acute or chronic distress or nutritional deficiency)

The animals will experience pain and discomfort after surgical procedures. The animals are given analgesics post operatively to counteract any unavoidable distress from the surgery. Following surgery there is an increased risk of infections, both around the craniotomy and inside the recording chamber. Inflammation and/or infection of skin margin around implant may occur. The wound and the interior of the recording chamber will be checked daily, and flushed with disinfectants

or other antibacterial solutions if necessary. If infections occur, antibiotics will be given to the animal as recommended by the veterinary staff of the CNPRC. The potential for seizures from lowering cannulae will be attenuated with Depakote and Valium, and the veterinary staff will treat brain edema if it occurs. Because water restriction could cause reduction in appetite and consequent weight loss, the monkeys will be weighed at least 5 days per week. One of the best indicators of animal health is active participation in behavioral tasks. Any changes in behavior during testing will be followed up with a consultation with the veterinary staff.

How will the signs listed above be ameliorated or alleviated? If signs are not to be alleviated or ameliorated by means of post-operative analgesics or other means, explain why this is necessary.

All monkeys are given routine administration of antibiotics and analgesics post-operatively.

Implants are monitored daily by investigators. Cylinders with open craniotomies are flushed with dilute Novasan and Betadine. Wound edges are kept clean and free from hair. Primate Center veterinarians will treat if necessary.

Diet supplements, fruit, vegetables will be given by investigators, in consultation with the Veterinary and Enrichment staff, to insure adequate nutrition. Periodic blood work is done by CRPRC to ensure the animals good overall health.

*Note: if any unanticipated adverse effects not described above do occur during the course of the study, a complete description of those effects and the steps taken to mitigate them must be submitted to the committee as an amendment to this protocol.*

Is death an endpoint in your experimental procedure?  Yes  No

*(Note: "Death as an endpoint" refers to acute toxicity testing, assessment of virulence of pathogens, neutralization tests for toxins, and other studies in which animals are not euthanized, but die as a direct result of the experimental manipulation). If death is an endpoint, explain why it is not possible to euthanize the animals at an earlier point in the study. If you can euthanize the animals at an earlier point, describe the clinical signs which will dictate that an animal will be euthanized.*

N/A

**j) Literature search for alternatives and unnecessary duplication:**

*This section is specifically required by Federal law. You are required to conduct a literature search to determine that either 1) there are no alternative methodologies by which to conduct this study, or 2) there are alternative methodologies, but these are not appropriate for your particular study. "Alternative methodologies" refers to reduction, replacement, and refinement (the three R's) of animal use, not just animal replacement. You must also show that the study is not unnecessarily duplicative of other studies.*

What was the date on which you conducted this search?

7/17/2002

List the databases searched or other sources consulted (there should be more than one). Include the years covered by the search.

Database Name	Years Covered	Keywords / Search Strategy
Medline	1966-2002	monkey, primate, physiology, neural activity, neural responses, learning, memory, paired associate
Science Citations	1966-2002	monkey, primate, physiology, neural activity, neural responses, learning, memory, paired associate

What were your findings with respect to alternative methodologies?

There are no current alternative methodologies to studying the electrical activity of single neurons in the alert macaque monkey. These measurements cannot be duplicated by computer models.

Has this study been previously conducted?

Yes  No

If the study has been conducted previously, explain why it is scientifically necessary to replicate the experiment.

A similar experiment has been conducted in young animals with very interesting results (Erickson & Desimone, 1999). We wish to compare the results obtained in young animals to those obtained in aged animals. In order to have tight scientific control over the experimental parameters, it will be necessary to include young animals as control.

**k) Disposition of animals:** At what point in the study, if any, will the animals be euthanized?

They will be euthanized once all available recording sites have been sampled in both hemispheres.

**l) Methods of euthanasia:** Even if your study does not involve killing the animals, you should show a method that you would use in the event of unanticipated injury or illness. If anesthetic overdose is the method, show the agent, dose, and route.

Species	Method	Drug	Dose (mg/kg)	route
Mulatta	Overdose	Pentobarb	60mg/kg	I.V.

**m) Surplus animals:** What will you do with any animals not euthanized at the conclusion of the project?

All animals will be euthanized.



Supervisors are responsible for insuring that their employees are adequate trained, both in the specifics of their job and in the requirements of the Federal Animal Welfare Act. EH&S offers free, basic wet labs in laboratory animal handling and techniques, and lecture format classes in the requirements of the Animal Welfare Act. To schedule a class for your unit, contact EH&S at 2-2364. Autotutorials are also available on the world wide web at <http://clueless.ucdavis.edu/>.

**Assurances for the Humane Care and Use of Vertebrate Animals:**

Principal Investigator's Statement:

I have read and agree to abide by the *UC Davis Policy and Procedure Manual* section 290-30 (Animal Use and Care). This project will be conducted in accordance with the *ILAR Guide for the Care and Use of Laboratory Animals*, and the *UC Davis Animal Welfare Assurance* on file with the US Public Health Service. (These documents are available from the Campus Veterinarian and at <http://ehs.ucdavis.edu/>). I will abide by all Federal, state and local laws and regulations dealing with the use of animals in research.

I will advise the Animal Use and Care Administrative Advisory Committee in writing of any significant changes in the procedures or personnel involved in this project.

Principal Investigator	Rank / Title	Date
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**Committee Use Only Below**

<p><b>** Conditions necessary for Committee Approval:</b></p>
<p>Final Disposition of this protocol:</p> <p>_____ Approved</p> <p>_____ Not Approved</p> <p>_____ Withdrawn by Investigator</p> <p>Date of Action: ____/____/____</p>

I verify that the Institutional Animal Care and Use Committee of the University of California, Davis, acted on this protocol as shown above.

Campus Veterinarian	Date
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