

PROTOCOL FOR ANIMAL USE AND CARE

Email to: campusvet@ucdavis.edu

CNPRC

EH&S USE ONLY

PROTOCOL: #10710

EXPIRES: 8/13/04

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	50	CNPRC

Project Title	Artificial Gravity and Light as Countermeasures for Circadian Dysfunction in Altered Gravitational Environments
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Overnight housing location::	CNPRC / CARU	Day use:	
Animals will be maintained by:	<input checked="" type="checkbox"/> Vivarium <input type="checkbox"/> Investigator (If investigator maintained, attach husbandry SOP's.)		

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.

Circadian rhythms of temperature, activity and sleep will be recorded from unrestrained monkeys via biotelemetry. This project will determine if the rhesus biological clock: 1) can be synchronized by a simulated martian lighting environment; 2) is affected by exposure to gravitational environments greater than Earth's (+G, produced via centrifugation); 3) is reset by exposure to acute pulses of light at different +G levels; 4) is reset by exposure to acute +G pulses under different illumination levels and 5) responds to +G fields differently at low or high illumination levels.

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.

Light dark cycles will include: constant light (LL), 24 hr (LD 16:8), 24.66 hr (LD:16h25m:8h12m; with either white or blue-green deficient spectrum), and 28 hr (LD:18h40m:9h20m; used to determine the period of the circadian clock). On the centrifuge, cages are set within recording modules. A nutritionally complete pelletized diet is provided through the Psychomotor Test System. Daily husbandry includes: cleaning/cage sanitation, urine pan change, food replenishment, lixit test, test of the PTS, determination of food and water consumption, and visual health check. Health status is logged and reported daily to CNPRC therapeutics.

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 type="checkbox"/> Clinician to treat	<input type="checkbox"/> Save for Investigator	<input 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 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:	NSBRI	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):	9191

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 ptillman@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 Circadian Timing System (CTS) is an important temporal organizer controlling both the physiology and behavior of organisms. The CTS has evolved to best adapt organisms to the 24-hour solar day experienced on Earth. The CTS is responsible for synchronizing an organism to the external 24-hour day (referred to as entrainment) as well as providing internal synchronization between an organism's physiological systems. Problems seen when living under non-24 hour schedules can include decreased performance, jet-lag, and physical dysfunctions associated with shift work. In addition, circadian dysfunction has a high comorbidity with some sleep and mental disorders, e.g. both endogenous and winter depression.

The most important time cue for the CTS is the external cycle of light and dark. Other environmental and social parameters have been shown to be effective time cues for the mammalian CTS. These include social interaction with conspecifics, cycles of food availability, access to running wheels and periodic exposure to an increased gravitational field produced via centrifugation. During a circadian study, it is vital to control all aspects of the subject's environment that may provide time cues to the CTS.

As we move towards longer-term space missions, humans will be exposed to non-Earth gravitational and lighting environments for increasingly longer periods of time. For example, a mission to Mars would encompass one to two years of travel combined with a stay on the planet of an additional 9 - 12 months. The light that reaches the martian surface is shifted towards the red end of the spectrum and is much lower in intensity than that seen on Earth. In addition, the rotation rate of Mars gives the planet a 24.66-hour day. The gravitational field on Mars is 0.38 G, i.e. 38% of Earth's gravitational field (1 G). All three of these factors (light intensity and spectrum, day length, and gravitational field strength) can affect the CTS. This program will conduct a series of experiments to determine if the martian lighting environment can effectively synchronize the circadian rhythms of male and female rhesus monkeys to a martian daylength. Further, we will determine the relationship between the period of the circadian pacemaker and the ambient force environment to predict whether or not the intrinsic period of the circadian clock will be shorter in martian gravity. If it is, this would prove an additional difficulty in achieving synchronization, also referred to as entrainment, to the martian day. We will also test proposed countermeasures designed to alleviate the circadian dysfunction, timed bright light exposure and timed +G exposure. We will examine the following hypotheses:

1. Rhesus macaques will not entrain (synchronize) to the martian solar day. This inability will be due to a combination of the non-24 hour day length (24.66 hours) and the significant reduction in blue-green light in the ambient light spectrum. We will also test the secondary hypothesis that there are gender differences in these responses.
- 2: Under a lighting environment proposed for the Martian Habitat (earth-solar spectrum, 100 lux intensity), some, but not all, rhesus macaques will be able to entrain to a day-length of 24.66 hours. There will be gender differences in these responses.

3. The addition of a daily evening bright light pulse will synchronize all rhesus monkeys to the proposed martian habitat environment. Such synchronization will ameliorate the physiological and behavioral dysfunctions that occur when the photic environment is insufficient to produce entrainment.
4. In hypergravity, the rhesus circadian period will change as a direct function of gravitational field strength. Further, in an increased force environment, there will be alterations in the phase-shifting response to a timed bright light pulse.
5. Exposure to acute pulses of 2.0G will phase shift the rhesus CTS.
6. Exposure to a +G environment will affect the normal increases in circadian period that occur as illumination levels are increased.

The rhesus, both as a diurnal species and as an established biomedical model with close taxonomic affinities to humans, provides a potentially unique model of human rhythms and sleep allowing detailed study of the underlying mechanisms and potential therapies for sleep and rhythm disturbances.

We propose to use telemetry to measure rhythms of brain temperature, activity, and sleep (EEG, EOG, EMG) in unrestrained animals.

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 type="checkbox"/> Food or water restriction | <input checked="" type="checkbox"/> Special diets; food or water treatment. |
| <input type="checkbox"/> Polyclonal Antibody Production ** | <input 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 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 checked="" 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.)

Subjects

Each subject will be fully trained to use the PTS system and have a telemetry transmitter surgically implanted prior to serving as a subject in an experiment. Each study will have an n of 8. Based on our previous experience, we anticipate that we may have to replace up to 2 individual rhesus in each study pool, bringing the total number of subjects used to 50. Individual animals will need to be replaced if: 1. they cannot be adequately trained to use the PTS system (this would be established prior to surgery), 2. the battery life of their transmitter has been exceeded and we have completed the experimental protocol, or 3. an animal develops a pathology, unrelated to the study, that makes it an unsuitable subject for the study. In the case of premature failure of the implant, when loss of a specific animal as a subject would require that the entire study be repeated, we would, with the concurrence of the CNPRC veterinary staff, explant and reimplant this animal. Based on our experience, 2 additional animals per study is a conservative estimate. Fewer animals may, in fact, be required. However, we have specified two to assure that the objectives of the study are not jeopardized.

Data Collected

In all experiments, brain temperature, activity and sleep (EEG, EOG, EMG) will be recorded from unrestrained animals by telemetry. Drinking will be recorded as contact with the lixit. Food intake will be recorded using the PTS. Daily food and water consumption will be recorded. Ambient temperature will also be recorded. Daily urine collections and observations will be made to monitor the menstrual cycles of the female subjects. Urine is collected in a pan placed underneath the cage. Total volume is measured and an aliquot frozen for later analysis.

PTS

The Psychomotor Test System (PTS) was developed at the Language Research Center at Georgia State University. Scientists at the Center perform social and cognitive studies on chimpanzees, bonobos and rhesus macaques. The director of the Center, Dr. David Washburn, is a consultant on these studies. The PTS consists of a series of computer generated video-based tasks that test various aspects of performance (such as hand-eye coordination and short term memory). Rhesus monkeys will be trained to use the PTS following protocols employed with success in our laboratory at UC Davis. The training program uses performance-based decision rules to manipulate task difficulty; the task does not increase in difficulty until the monkey has achieved a set level of success. Naive monkeys learn to control the movements of a computer-generated cursor and to respond to computer-graphic stimuli. Once the joystick and computer tasks are made available to a monkey, the animal can work or rest when it wants. Nutritive rewards (fruit-flavored, nutritionally complete food pellets) are delivered upon successful completion of each trial. The monkeys are not deprived of food (i.e., reduced in body weight to make them hungry) or water during training or testing. The PTS is available *ad lib* to the animals and fully satisfies their nutritional requirements. During training and experiments, the number of pellets consumed each day is recorded. If the amount of food obtained via PTS does not meet the caloric requirements of an animal, adequate supplemental food is provided during daily animal husbandry. Numerous experiments have demonstrated not only that monkeys can learn the PTS tasks in this way, but also that access to the PTS tasks constitutes a powerful form of environmental enrichment that serves to support the psychological well-being of research primates. In short, it is likely that PTS training and testing provide generalized benefits beyond the important psychological data they provide. All subjects are thoroughly trained in PTS use prior to bioinstrumentation. Any candidate that does not successfully complete PTS training will not serve as a subject in these experiments.

Environmental Enrichment

PTS has been shown to provide environmental enrichment to rhesus macaques. Studies have shown that rhesus monkeys exhibit a preference for PTS over other forms of environmental enrichment. Rhesus monkeys devoted on average 9 hours per day to PTS tasks, even when other attractive manipulanda and activities were present. PTS reduced behavioral indications of psychological distress, such as stereotypy, over-grooming and aggression, to a greater extent than puzzle boxes, swings, balls or the presence of a compatible conspecific (70% reduction for PTS vs. 20% and 40%, for manipulanda and pair-housing, respectively). Animals continued to access PTS even when given free access to food pellets. When given the choice of performing PTS tasks for pellets or receiving pellets freely for 30 minutes, but with PTS tasks denied them during this time, animals opted to perform tasks for pellets. The results of all of these studies reveal that food alone does not drive PTS performance by rhesus monkeys. In addition, PTS is a task oriented feeding method with several key advantages over other task-

based feeders (namely variety of task, choice and control by animals, and changeable level of task difficulty). Animals are fully provisioned, whether or not they use the PTS.

Social Enrichment

During a circadian study, it is vital to control all aspects of the subject's environment that may provide time cues to the CTS. While, the most important time cue for the CTS is the external cycle of light and dark other environmental and social parameters have been shown to be effective time cues for the mammalian CTS. These include social interaction with conspecifics. Because of this we request an exception to the requirement for visual social contact during the periods of data collection for these experiments. Animals will be in vocal communication at all times.

Bioinstrumentation

Instrumentation will include implanting biosensor leads (24 cm) for recording of electroencephalogram (EEG), electrooculogram (EOG), and electromyogram (EMG), and a brain temperature sensor (3 cm) and associated telemetry transmitters (transmitter = 5.7 x 2.8 x 0.92 cm, battery = 5.1 x 2.8 x 0.92 cm) and antenna (30 cm). All telemetry is fully implantable and no further maintenance should be required. The transmitter contains a magnetic switch and can be turned off when data is not being collected in order to conserve battery life.

Surgical Procedures. Monkeys will be fasted overnight prior to surgery. Monkeys will be instrumented under a surgical level of anesthesia. Implant surgeries and procedures will be performed at the California National Primate Research Center (CNPRC).

For the surgical procedure, the monkey will be preoperatively medicated with ketamine (10 mg/kg) intramuscularly (IM), atropine (0.04 mg/kg subcutaneously (SC) or IM) and the antibiotic cefazolin (20 mg/kg) IM. The subjects will be intubated and maintained at a deep surgical anesthetic level by a trained anesthetist using isoflurane gas at a maximum allowable concentration (MAC) of approximately 1.25%. Intravenous fluids will be administered by the attending veterinarians, typically at a rate of 10 ml/kg/hour. Following induction and throughout surgery and post-anesthetic recovery, respiration, heart rate and body temperature will be monitored by conventional means with the animal remaining on a warm pad until sufficiently recovered to be placed in a recovery cage. At this time, intravenous fluid therapy will be discontinued. The endotracheal tube will be removed when the gag reflex has recovered. Animals will be observed until fully recovered by the attending veterinarians and animal care staff. Oxymorphone will be used as a post-operative analgesic (0.15 mg/kg TID for 2 days). Animals will be monitored carefully during the 48-hour period immediately following surgery by the attending veterinarian and members of the CNPRC veterinary staff. Skin incisions will be checked daily until healed. Antibiotics will be given post-surgically (e.g. cefazolin 20 mg/kg TID for 5 days).

Biosensor Implant: The telemetry transmitter will be placed between muscle layers in the abdominal wall and cranial leads will be routed subcutaneously. First, a 5 cm skin incision will be made at the desired location. A midline incision is made along the sagittal plane of the scalp, and the underlying bone exposed by blunt dissection. The telemetry transmitter and battery will be placed contralaterally within the abdominal musculature. Cranial leads from this transmitter (EEG, EOG, EMG, brain temperature) will be routed subcutaneously.

Cranial implants: The head is restrained in a stereotaxic device. The stereotaxic coordinates of the brain probe and 3 EEG locations marked lateral to the midline over the midbrain (temperature), and frontal, parietal, and occipital areas (EEG). Small craniotomies are made with a dental burr. The brain temperature probe is inserted through the predrilled hole into white matter near the thalamus and secured with dental acrylic. Holes are tapped and cleared. Bone screws passed through the EEG electrodes are inserted to contact the dura and secured with dental acrylic.

EOG electrodes: EOG electrodes are positioned lateral to and above and below the orbital canthus and secured with miniature bone screws.

EMG electrodes: After removal of the stereotaxic frame, EMG electrodes are placed on the posterior neck musculature and secured with sutures.

Transmitter placement: After all cranial leads are secure, the transmitter is secured within the abdominal muscle wall with sutures.

Conclusion: At the conclusion of all implant procedures, skin incisions will then be closed with a continuous intradermal pattern of 4-0 vicryl suture. Subjects will remain in the hospital for two weeks and implant sites will be assessed visually on a daily basis by veterinary staff and subsequently daily during health checks. Any adverse observations will be reported to the veterinary staff.

Centrifuge

The large diameter centrifuge used to create the +G fields has modules that each contain a standard rhesus cage. The modules have one degree of freedom of movement; during rotation they swing out, producing an increased force vector directed perpendicularly down through the floor of the animal's cage (i.e., in the same direction as normal gravity). In addition to providing control of the lighting environment and ventilation, these modules also act to shield the animal from visual cues about the rotating environment. At the onset of centrifugation, animals may experience a transient (1-2 day) anorexia. Our study design incorporates a step adaptation to the +G environment. Animals are exposed and adapted to 1.5G prior to 2.0G exposure. This design minimizes the adaptation stress. Any experiment requiring exposure to a gravitational field greater than Earth's 1 G will be performed on the centrifuge. Experiments not requiring +G exposure can either be performed on the centrifuge or at the CNPRC.

Cage Sanitation

In studies of rhesus circadian rhythms, light cycles and other environmental time cues need to be carefully controlled. It is vital that the animals be disturbed as little as possible and only at restricted times. The procedure we have used for cage sanitation is two-tiered. We employ CoveragePlus (1:256 dilution) as a chemical disinfectant in combination with daily pressure rinsing of the cages and waste pans. At four-week intervals, the monkeys are placed in holding cages and the home cages are sterilized with bleach solution and samples taken for microbiological testing. Cages are steam cleaned between studies (30 – 120 day interval). Microbiological testing has shown that the cages remain clean and sanitary using these procedures. During experiments, all animal husbandry is provided by personnel thoroughly trained in these procedures.

Experiments

Experiments will be performed to determine if rhesus will entrain to light in the martian environment. We will examine the effects of the spectral composition of martian light and the martian daylength on the CTS, and develop projections of the influence of martian gravity level. We will also determine the effect of gravity level on circadian period, leading to predictions of the effect of martian gravity on entrainment. In addition, we will test the effects of two proposed countermeasures, +G pulses and light pulses, on the rhesus CTS. PTS access will be *ad lib* during light periods, and water will be available at all times. Ambient temperature will be regulated at 26 ± 1 °C. Lighting will be provided by either cool-white fluorescents, daylight halogens or from blue-green deficient lamps. Telemetry implants will be used to monitor brain temperature, activity and sleep states. The investigators will have access to real time physiological data at all times. Sensor calibration is performed with known temperature and voltage both prior to implant and post explant.

Experiment 1

Eight male rhesus will serve as subjects for Experiment 1. These data will be compared with those collected previously from female rhesus using the same experimental protocol in order to determine if there is a gender difference in the responses of the rhesus CTS to exposure to the martian lighting environment. The females evidenced no adverse behavioral or physical effects from exposure to any of the experimental lighting conditions. Experiments 1A, 1B and 1C will be performed consecutively, with a 2 week return to baseline LD 16:8 in between each data collection period. Cages will be steam cleaned during this time.

Experiment 1A. This experiment will determine if the martian lighting environment provides sufficient photic information to entrain (synchronize) the primate CTS. Data will be recorded from 8 male rhesus maintained under the martian spectrum at 100 lux intensity, and in a martian day length (24.66 hours; 16 hours and 25 minutes of light followed by 8 hours and 12 minutes of darkness) for one month, or until entrainment has been demonstrated. PTS will be available throughout the light period. Water will be available *ad libitum*. Daily animal husbandry will be performed on an alternating early/late schedule that is outside the circadian range. Brain temperature, activity, and drinking will be recorded continuously. Biweekly 48-hour sleep recordings will be made for each subject.

Experiment 1B. This experiment will determine if the lighting environment proposed for the Martian Habitat is an effective synchronizer of the rhesus CTS, and if long-term exposure to the habitat environment adversely affects the CTS and performance in rhesus. We will also determine if there is a gender difference in these effects.

The protocol from Experiment 1A will be used. The only difference being that the light spectrum during the L portion of the lighting cycle will be that of solar illumination on Earth. The intensity will remain at 100 lux.

Experiment 1C. This experiment will determine if the addition of an appropriately timed light pulse will allow entrainment to the 24.66 hour martian day. The protocol from Experiment 1A will be used. The light spectrum will be that of solar illumination on Earth ($L = 100$ lux). In addition to the background LD cycle, each animal will be exposed to a 1-hour bright light pulse (1800 lux) early in the night.

Experiment 2

This experiment determines how circadian period changes with G level in order to create a predictive model for the effects of the martian gravitational environment on the period of the primate CTS. This experiment has already been performed using male rhesus as subjects. Aside from a brief anorexia at the onset of centrifugation and at the step change from 1.5 to 2.0 G, these male subjects evidenced no adverse behavioral or physical effects during the study. For this study, female ($n = 8$) rhesus monkeys will be studied at three G levels: 1G (normal earth gravity), and at 1.5 and 2.0 G on a large diameter animal centrifuge. To determine circadian period at each G level, we will use a “forced desynchrony” protocol. In an environment without time cues, the period of a circadian rhythm is determined by the circadian clock, or pacemaker. However, the period of the clock is affected by the ambient light intensity. The “forced desynchrony” protocol has been developed to avoid the confounding effect of light intensity and allow determination of the true period of the clock. In the “forced desynchrony” protocol, a light-dark cycle is presented that is too long to synchronize the circadian clock. Behavioral rhythms, such as the sleep-wake cycle or feeding, can be “forced” to conform to the light-dark cycle, while physiological rhythms, such as the body temperature rhythm, express the period of the clock. This method avoids constant light exposure, minimizing influences of illumination intensity on period. Further, direct responses to light, for example, the tendency to increase body temperature and alertness, are distributed across the circadian cycle. We have previously employed this protocol successfully with rhesus monkeys and have seen no adverse health effects in the subjects. Animals will be housed at 1.0 G level for 30 days in a 24 hr LD 16:8 cycle. Animals will then be exposed to a 28 hour light-dark cycle, with a 2:1 ratio of light to dark (LD 18.67:9.33, in hours), with food available (through PTS) during the L portion of the LD cycle. Data will be recorded as described in Experiment 1. The animals will be exposed to this LD cycle for 30 days. This protocol will be repeated at 1.5G and at 2.0G beginning with acclimation to the standard LD 16:8 cycle.

Experiment 3

Eight male rhesus will serve as the subjects for Experiment 3A and an additional eight male rhesus will serve as subjects for Experiment 3B.

Experiment 3A. The ability of timed light pulses to phase-shift the rhesus CTS will be tested at several G-levels (1.0, 1.5 and 2.0). Animals entrained to LD 16:8 will be released into constant light for 14 days. The period and phase of the circadian rhythms will be determined. These data will serve as the control. Animals will again be entrained to LD 16:8 and then released into constant dim light (13 lux). During the first 24 hours of LL, a two-hour pulse of bright light (~1000 lux) will be presented early in the animal’s night. A light pulse presented at this time of day should cause a phase delay in the CTS. Data will be recorded for two weeks. The period and phase of the circadian rhythms will be determined and compared with the control. The animals will again be entrained to LD 16:8 and released into LL, but this time the bright light pulse will be given late in the animal’s night, a time of day when a light pulse should produce a phase advance of the CTS. The entire protocol will be repeated at 1.5G and at 2.0G produced via centrifugation. Cages will be sanitized with bleach during each LD 16:8 baseline.

Experiment 3B. The ability of timed +G pulses to phase shift the rhesus CTS will be tested. The protocol described for Experiment 2A will be followed with the following changes: the animals will remain at 1G, one hour pulses of 2.0G will be substituted for the light pulses and the effects of the 2.0G pulses will be tested in LL of 13 lux and LL of 200 lux. We are using these two levels of light intensity because the period of the circadian clock is affected by light intensity. The period of the circadian rhythms should be longer in LL 200 lux than in LL of 13 lux.

Experiment 4

In constant light, as illumination intensity increases, the period of the circadian pacemaker also increases. This experiment will determine if exposure to an increased gravitational field alters this response. Eight male rhesus

monkeys will serve as subjects of this study. Data will be recorded for two weeks in LD 16:8 at 1G. The animals will be released into constant dim light (13 lux) and data will be recorded for an additional two weeks. The animals will be returned to baseline LD 16:8 conditions for two weeks and then again released into constant light (200 lux). Data will again be recorded for two weeks. This protocol will be repeated at 1.5G and again at 2.0G. Cages will be sanitized with bleach during the LD 16:8 baseline intervals.

Post-Experiment

Implant Removal Surgery. Pre-surgical preparation will be done as for the implant surgery, above. A cranial incision will be made to expose each lead. The EEG and EOG electrodes will be detached from the bone screw anchors and the bone screws removed. The dental acrylic securing the brain temperature probe will be removed and the probe extracted. Following removal of EEG and EOG electrodes, and the brain temperature probe, holes will be filled with bone wax. An incision is made over the transmitter and battery sites. Any connective tissue anchoring the leads is dissected away and the leads withdrawn through the abdominal skin incision. The transmitter body is freed from any connective tissue overgrowth and removed. All incisions are sutured. Post surgical treatment will be as for the implantation surgery. Observation and monitoring of animals will be done for at least 2 weeks after implant removal.

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
1	PTS Training	10	1
1	Implant Surgery	10	3
2	PTS Training	10	1
2	Implant Surgery	10	3
2	Exposure to 1.5G and 2.0G via centrifugation	10	2
3A	PTS Training	10	1
3A	Implant Surgery	10	3
3A	Exposure to 1.5G and 2.0G via centrifugation	10	2
3B	PTS Training	10	1
3B	Implant Surgery	10	3
3B	Exposure to 1.5G and 2.0G via centrifugation	10	2
4	PTS Training	10	1
4	Implant Surgery	10	3
4	Exposure to 1.5G and 2.0G via centrifugation	10	2

Categories of invasiveness

Category	Description
1	Little or no discomfort or stress Examples: 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 Examples: 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 Examples: 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 Examples: 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?

An n of 8 within each group (males and females) will provide for statistically significant measurements (ANOVA, Student's t-test). The rhesus is an established human surrogate, with robust circadian rhythms and consolidated daily sleep very similar to humans. Based on our previous experience, we anticipate that we may have to replace up to 2 individual rhesus out of the 8 total subjects during the completion of an experiment. This would increase the total number of subjects used to 10. PTS training takes place prior to any surgical procedures. An animal that did not successfully complete PTS training would not be used as a subject in these experiments. A large literature exists for this species to aid interpretation of our results.

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

Building: 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 mg/kg	IM	Pre-operatively
Rhesus	Atropine	0.04 mg/kg	SC or IM	Pre-operatively
Rhesus	Cefazolin	20mg/kg	IM	pre-operatively; post-operatively (TID for 5 days)
Rhesus	Isoflurane	To effect	Inhal.	Peri-operatively
Rhesus	Oxymorphone	0.15 mg/kg	IM	Post-operatively, TID for 2 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?

ECG, respiratory rate, body temperature

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

To maintain surgical plane

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)

There may be pain or discomfort from the surgical procedures. These will be alleviated with anesthetics and analgesics. The use of telemetry allows us to perform this study on unrestrained animals. As with any surgical procedure, there is a risk of infection. Having a fully implantable telemetry unit with no percutaneous leads reduces the risk of post-surgical infection. Because there are no percutaneous leads, the continuous use of a jacket will not be required. This both reduces the possibility of skin erosion (caused by friction between the animal and the jacket) and allows for easier inspection of the implant sites. Adverse signs would include any indication of the presence of infection (swelling, redness, discharge) or separation or erosion of the skin over an incision site or over the implant.

With the exception of the +G pulses, we have previously used all of the described experimental procedures (constant light, forced desynchrony, light pulses, 1.5G and 2.0G exposure) without adverse effects in long-term studies with rhesus. Rhesus evidence no behavioral or physical problems during exposure to constant light of the intensities to be used in these studies, nor to the forced desynchrony protocol or other light-dark cycles. All of these lighting protocols are commonly used in circadian studies. Upon the initiation of centrifugation, animals may experience a brief (1-2 day) anorexia that appears tied to vestibular adaptation to the new force environment. We have used +G pulses in other species without adverse effects and do not anticipate adverse effects from acute exposure to 1 hour of +G in rhesus.

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.

Each potential subject undergoes a pre-screening exam and must meet criteria established by CNPRC veterinarians & CARU scientists to become an experimental candidate. Criteria include body size, examination of the skin and implant sites and a review of the animal's behavioral history to ensure that there are no behavioral problems.

Surgery is performed under general anesthesia and post-operative analgesics are also used. Appropriate preventative antibiotics will be administered to reduce the risk of infection. Animals spend two weeks in post-surgical recovery in the CNPRC hospital with daily monitoring by veterinary staff. All implant sites (transmitter, battery, and all lead sites) are assessed visually on a daily basis during the animal health check. In addition, the implant sites are palpated by CNPRC veterinary staff when the animals are removed from the cage for cage sanitation by either bleaching or steam cleaning. A CNPRC veterinarian is consulted if any adverse signs are observed. If an adverse condition cannot be successfully treated with the implant *in situ*, the implant will be completely removed surgically and the animal treated with antibiotics as deemed appropriate by the CNPRC veterinary staff.

Having the rhesus adapt to 1.5G prior to exposure to 2.0G minimizes the period of anorexia. There has been no emesis noted upon exposure to 1.5G or to 2.0G after adaptation to 1.5G. To date, we have never had a rhesus monkey that did not adapt to +G exposure, however, if an animal were unable to adapt to the +G environment, it would have to be removed from the study.

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 [x] 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.

j) Literature search for alternatives and unnecessary duplication:

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

UC Davis provides on-line access to a number of databases that can be used to search for alternatives. Visit

http://trc.ucdavis.edu/jawelsh/Databases_Med_Vet_Researchers.htm (email: jawelsh@ucdavis.edu)

or http://www.vetmed.ucdavis.edu/Animal_Alternatives/main.htm (email: mwood@ucdavis.edu)

What was the date on which you conducted this search?

6/25/2003

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*	1966-2003	rhesus and sleep, light, and gravity
Medline	1966-2003	telemetry and alternatives
Medline	1966-2003	rhesus and circadian rhythms
Biosis	1966-2003	telemetry and alternatives
		*Spaceline is now a subset of Medline

What were your findings with respect to alternative methodologies?

Biotelemetry provides the most benign method for performing this study. The use of telemetry obviates the need for restraint and tethers. The telemetry unit is fully implantable, eliminating the risk of infection at lead exit sites as in tethered animals.

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.

This is an on-going study examining the relationship between the rhesus CTS, light and gravity. Experiment 1 has been completed using female subjects and Experiment 2 using male subjects. We are going to repeat Experiment 1 using male subjects and Experiment 2 using female subjects in order to determine if there is a gender difference in the responses. The other experiments described in this protocol have not been previously performed.

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

These studies are not terminal. The animals will be highly trained and conditioned, and their implant design should allow for their continued use in future studies. Evaluation of each animal for future use will be done by the investigators and the CNPRC veterinary staff. Animals not considered appropriate for continued use will be euthanized.

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
Rhesus	Anesthetic overdose	Pentobarbital	60 mg/kg	IV

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

These animals will be highly trained and represent a significant investment in time and dollars. We would like to preclude a situation in which we are forced to euthanize a healthy and useful animal. If the animal is no longer a suitable candidate for one of our studies, it may still be valuable to another investigator. At the conclusion of our study, the animals will be released for review. Their final disposition will be decided on a case by case basis and will be one deemed appropriate by the CNPRC and in accord with the Animal Welfare Act. The only situation, in which we would consider reimplantation, would be in the case of premature failure of an implant, when loss of a specific animal as a subject would require that the entire study be repeated. In such a situation, given the concurrence of the CNPRC veterinary staff, we would want to explant and reimplant this animal.

swings, balls or the presence of a compatible conspecific (70% reduction for PTS vs. 20% and 40%, for manipulanda and pair-housing, respectively). Animals continued to access PTS even when given free access to food pellets. When given the choice of performing PTS tasks for pellets or receiving pellets freely for 30 minutes, but with PTS tasks denied them during this time, animals opted to perform tasks for pellets. The results of all of these studies reveal that food alone does not drive PTS performance by rhesus monkeys. In addition, PTS is a task oriented feeding method with several key advantages over other task-based feeders (namely variety of task, choice and control by animals, and changeable level of task difficulty). Animals are fully provisioned, whether or not they use the PTS.

B. Duration: at all times during data collection

3/1/00

Date: Mon, 07 Jul 2003 16:05:16 -0700
 Subject: Protocol 10710 - Attn: Sharon
 From:
 To:
 CC:

Hi -

I've revised the protocol to answer your questions. I've also included the answers below. If any others come to mind, please let me know.

Thanks,

>Protocol 10710 ()

>Since this is an earlier version of the protocol, a number of sections in your protocol may need revising. In the future, please download the most current version of the protocol from the ehs.ucdavis.edu website under Animal Care and Use.

It is now on the new form.

>

>1. You have listed the CNPRC as the only housing area. Since you have described procedures that appear to be conducted on the centrifuge, do you now have a centrifuge at the primate center or do you need to add CARU to the protocol. Also, the "day use only" box now reads "day use". Will some of your work be conducted in a lab or non animal area? We are now creating a database of study areas above and beyond the animal rooms because we were cited by AAALAC for not visiting study areas. The "day use" box will hopefully collect that information.

We will be conducting the experiments at CARU and CRPRC. All experiments will be conducted in animal space.

>

>2. In section c, what do you mean by "entrainment"? Please clarify.

Entrainment is synchronization of the circadian clock to the external 24 hour light-dark cycle. I stuck this definition in a couple of places, since the term is unique to circadian research.

>

>3. In section c, you use a number of acronyms. Please qualify what you mean by Lm, D, G, h, m and lx.

I defined these where I found them; L is light, D is dark, G is the earth's gravitational field strength, h is hour, m is minute, lx is lux (measurement of light intensity, there are approximately 10 lux in one foot-candle)

>

>4. If you are using the same 16 animals, what is your time frame between experiments?

The experiments are now 1 (A, B, C), 2 (A, B), and 3. I've separated the subjects into the distinct experiments in table d. I also talked to Donna, and have now included the total number of animals to be used over three years, not just the number per experiment.

>

>5. Which experiments are to be conducted on the centrifuge?

We can do them all on the centrifuge since we use identical caging at the CRPRC and on the centrifuge, but any that don't require the +G environment can be done in 3030.

>

>6. In experiment 6, please explain what you mean by housing animals at one of three G levels and do you have all 16 animals at all 3 G levels? If they are at each level for 30 days, do you have a transition time between the times on the centrifuge?

The return to LD 16:8 allows for a reacclimation between the 28 hour day schedule used to determine the period of the circadian clock.

>

>7. In the "centrifuge" paragraph, you state that the cages will be steam cleaned between studies. What is the approximate timing of the cleaning or the studies?

30-120 days

>

>8. In section d, you list 16 animals, but have 24 on page 1 and discuss the need for 8 additional animals in section c, but in section e, you justify only the 16 animals. Please revise section e to provide justification of the 24 requested.

OK, fixed this.

>

>9. In section g, the oxymorphone was not included. What other agents might you be using that were included in the text but not in the list of agents? Please include ALL agents used on your study.

>

Just oxymorphone left out of table. I put it in.

>10. In section i, second box, you refer to the use of analgesics peri-operatively but no where else is this discussed. What agent will you use and when (dose, route and frequency).

I was thinking of the pre-operative ketamine, but that is very short-acting (and not really an analgesic) so I've just described the post-operative analgesics.

>

>11. In section j, last box of lit search, you have not provided a scientifically based reason for continuing to conduct this work. Please revise to explain why it is scientifically necessary to replicate the >experiment.

We've done experiment 3 with male subjects and will be repeating it with female subjects.

Date: Thu, 17 Jul 2003 15:18:54 -0700
 Subject: Re: More committee questions: Protocol 10710
 From:
 To:

Hi -

I've started to put these in the protocol, but I thought I'd send my answers to you to see if you find them complete. I have also attached the chart used to determine if daily food supplementation is necessary.

> Protocol 10710 ()

>

>> 1) Under c) there is mention that animals that develop pathologies that render them unsuitable for the project would be removed from study. There is no mention of these potential pathologies in the section on adverse effects . . . The committee is assuming that you were considering that these would be unforeseen medical conditions unrelated to the project and study manipulations. If there are pathologies that would develop as a result of the experimental manipulations, please provide a list with the steps to be taken to alleviate them and/or endpoints at which time animals would be removed from study at the discretion of the CRPC vet staff.

The committee is correct in assuming that the pathologies that would necessitate removing an animal from the study would be unexpected medical conditions unrelated to the study. We do not anticipate that any of the study methodologies will result in an animal developing a pathology.

>>

>> 2) Under the adverse effects section, the only things discussed are those associated with the surgery and implantation.

- a. In your experience, are there any potential adverse effects of the environmental manipulations including the low light periods?

➤

In all our work with rhesus monkeys we have never had an adverse effect resulting from light exposure, either continuous dim light, continuous bright light or bright light pulses.

- b. Do animals develop physical or behavioral problems associated with the manipulations?

➤

Manipulation of the light environment does not adversely affect the subjects. At the initiation of centrifugation, there is usually an initial period when food and water consumption are decreased. This can be accompanied by a loss of circadian rhythms and a suppression of activity. This is the normal response in every species studies thus far. In addition, primate subjects (humans, rhesus) can also exhibit symptoms of nausea (remaining horizontal on the cage floor, sometimes vomiting). We minimize this response having a period of adaptation to 1.5G prior to exposure to 2.0G (or, as in these studies, by performing the study at 1G, then 1.5G then 2.0G). The initial responses to 1.5G are slight and adaptation occurs within a period of 1-3 days, depending on the subject. Once an animal is adapted to 1.5G, increasing the acceleration field to 2.0G causes no adverse responses.

- c. How is this monitored for and how alleviated, if discovered? For example, if the animals have to use the PTS to access food (needs to be clarified - see below) and if their performance deteriorates due to the environmental changes, will they go off feed with subsequent deterioration of body condition and weight loss or failure to gain; do they develop signs of lethargy or depression or other behavioral signs of distress
 Can some limits to allowable weight loss, body condition loss, or failure to gain be set?

➤

Except for the initial response to onset of +G, we have not seen any deterioration in performance or behavioral signs of distress in response to any experimental manipulation. However, one of the objects of this study is to determine if exposure to the Martian lighting environment will adversely affect circadian entrainment and performance. What we hope to see in these studies is a decrease in performance that results in a decrease in the percent correct on a task, with no concomitant food deprivation to the animal.

Food consumption for the previous day is calculated for each animal at the time of daily husbandry. We have a chart that lists minimum and maximum food requirement by body size. If an animal's food consumption falls below

the minimum necessary, it can be supplemented with either additional pellets or with Purina Monkey Chow biscuits. Each PTS system is tested daily to ensure that it is performing properly.

>

>> 3) Under section c) "experiments", it is stated that food is available ad lib during light periods. Under experiment 1A, it states that food is available ad lib via PTS. Does this mean that the animal has to "work" to >> attain food? If the animal's ability to work deteriorates, what would happen in this case? Please clarify what ad lib food via PTS means. Ad lib is usually used to mean that animals have free access to food to satisfy their appetite - it does not mean that they have to perform or be able to perform "work" to get it.

PTS access is unrestricted at any time the lights are on. In addition, each successful completion of a task is rewarded with a pellet. Daily food consumption is determined and supplemental food is provided if necessary. Each PTS task tests different aspects of performance - for example, short term memory or hand-eye coordination. In addition, the tasks differ in level of difficulty. The tasks are presented in a suite of 5 and the subject picks which task it will perform. A set of 5 trials of the selected task is presented, after which the animal can choose another of the tasks to complete, or it can simply wait and the entire system resets, again presenting the suite of 5 tasks. A 6 kg animal needs to complete 670-955 tasks successfully to meet its nutritional requirements. It is not unusual for an animal to complete 1500 or more trials in a 24 hour period.

>>

>> 4) The study groups are listed, but it is still difficult to trace the time line of manipulations for animals within each group. Please provide a time line (estimated) for each group of animals that includes the periods of the different manipulations and the "rest" periods in between, followed by the estimated time when animals would be explanted? The committee realizes that this needs to be somewhat flexible to allow for individual differences for animals to adapt to the different conditions, but we should know the extent of time proposed for the use of each animal. We anticipate that one group of animals will complete all parts of experiment 1, another all parts of experiment 2 and a third experiment 3.

(I'll have to work this up in more detail).

>>

>> 5) There appears to be a typo under 2B where it is stated that the protocol for 2B will be followed with the following changes - do you mean that the protocol for 2A will be followed with the . . . changes?

Yes. This has been corrected.

>

>

>