



Department of Energy
Brookhaven Site Office
P.O. Box 5000
Upton, New York 11973

FEB - 8 2010

Mr. Ian Smith, M.A.
Research Associate
Laboratory Investigations Division
People for the Ethical Treatment of Animals
501 Front Street
Norfolk, VA 23510

Dear Mr. Smith:

SUBJECT: U.S. DEPARTMENT OF ENERGY (DOE) FREEDOM OF INFORMATION ACT (FOIA) REQUEST #CH-2010-00797-F

I am the authorizing official responsible for making the determination required by Section 1004.5(b) of DOE regulations found at 10 CFR Part 1004, which implements the FOIA, 5 U.S.C. 552.

In your original FOIA request you were seeking, "copies of all documents – including completed protocols, animal use records, veterinary records, meeting minutes, reports, and memos – pertaining to radiation experiments carried out on nonhuman primates at NASA's Space Radiation Laboratory Brookhaven National Laboratory (BNL) (2009-present)."

On January 21, 2010, in your e-mail to DOE FOIA Counsel, Megan Mikhail of the DOE Office of Science-Chicago Office (SC-CH), you narrowed your request to exclude "the names of the individuals as well as phone numbers, fax numbers, email addresses, signatures and initials." On January 29, 2010, in your phone conversation with Ms. Mikhail, you confirmed that you are searching for documents pertaining to any radiation experiments that have been conducted or are being planned at the Space Radiation Laboratory.

Be advised that we searched the BNL for records responsive to your narrowed request and located the enclosed responsive documents. Any additional redactions made are reflective of nonresponsive material. Therefore, this response represents our full release of responsive documents.

You would be categorized as an "Other" requester, and as such related costs for search and duplication of records would normally be charged. However, after review of the criteria you supplied in your request to support a fee waiver, on January 20, 2010, DOE SC-CH granted your fee waiver request, and accordingly, there are no fees associated with your request at this time.

Mr. Smith

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If you have questions regarding this response, please contact DOE SC-CH FOIA Officer Miriam Legan, at (630) 252-2041 or via e-mail at miriam.legan@ch.doe.gov.

Sincerely,



Michael D. Holland
Site Manager

Enclosure:
As Stated

cc: M. Legan, SC-CH, w/o encl.
M. McCann, BSA, w/o encl.



Building 490
P.O. Box 5000
Upton, NY 11973-5000
Phone [REDACTED]
Fax [REDACTED]
[REDACTED]@bnl.gov

managed by Brookhaven Science Associates
for the U.S. Department of Energy

Memo

* * *

DATE: January 11, 2010

TO: [REDACTED]

[REDACTED]
2010.01.11
13:55:09 -05'00'

FROM: [REDACTED], Chair, Institutional Animal Care and Use Committee (IACUC)

SUBJECT: IACUC Protocol 405 "Ground-Based Studies in Neurobehavioral Biology"

At the 01/07/10 IACUC meeting, the IACUC determined the initial application requires the following modifications:

1. The custom made chambers for irradiation must be discussed with NSRL and BLAF staff prior to being sent to BNL;
2. **Section C.1:** Should clarify whether or not acute effects from radiation are expected and discuss any previous primate data to support this; Correct spelling in second sentence "six monkeys *per* trip; Clarify whether six monkeys at a time are brought to and from the NSRL or whether they are staggered depending on when they are being irradiated; The radiation dose chosen must be justified in terms of its relevance to space travel;
3. **Section E.1:** Should be changed to 36;
4. **Section H.1:** The box for "Radioactivity" should be checked.

Please respond to [REDACTED] Bldg. 490.

Minutes of the BNL Institutional Animal Care and Use Committee
January 7, 2010

Present: [REDACTED]

Secretary: [REDACTED]

Guest: [REDACTED]

Excused: [REDACTED]

The meeting was held in the 490D Conference Room in Building 490 and was called to order at 1:05 pm by [REDACTED] Chair.

Actions Taken Since the Previous IACUC Agenda:

IACUC Protocol [REDACTED]

IACUC Protocol [REDACTED]

IACUC Protocol [REDACTED]

IACUC Protocol [REDACTED]

Old Business

New Business

IACUC Protocol 405 "Ground-Based Studies in Neurobehavioral Biology", PI: [REDACTED] Initial application discussed. The Institutional Official stated there is a separate scientific review committee (SACR) that reviews all applications for the NSRL but this protocol hasn't gone through that review process yet. He reiterated to the IACUC that this protocol is a sensitive topic due to the media attention it has gotten since it proposes to irradiate squirrel monkeys but that it should not be treated differently from other protocols. BNL management will make the final determination as to whether it is appropriate to conduct the study at BNL. A member questioned whether the radiation dose would affect the animals adversely. The NSRL liaison responded that it was a low dose and there should be no short term effects. There was a discussion on the holders for animals and how big they are in relation to the animals. The investigators should clarify this with the BLAF Manager. It was noted the total number of animals in E.1 does not match the total justified and should be changed to 36. It was not clear in the protocol whether the animals would be brought six at a time to the NSRL or whether their delivery and return to BLAF would be staggered. It would be better for the animals if they could be brought back once the radioactivity has decayed rather than staying at the NSRL until all studies are done for that group. The investigators will be asked to clarify this issue. It was noted "radioactivity" should be checked in Question H.1. A member stated the investigators should justify the radiation doses of 0.5 and 1.0 Gy. The investigators will be asked to submit previous data on monkeys, whether acute or chronic, and describe the relevance to the NASA missions. The investigators should also confirm

IACUC Meeting 01/07/10

whether there will be acute affects from the radiation at BNL. The initial protocol was approved for one year by a vote of 8 yes, 0 no pending the following modifications: The custom made chambers should be discussed with NSRL and BLAF staff prior to being sent to BNL; **Section C.1:** Should clarify whether or not acute effects from radiation are expected and discuss any previous primate data to support this; Correct spelling in second sentence "six monkeys *per* trip; Clarify whether six monkeys at a time are brought to and from the NSRL or whether they are staggered depending on when they are being irradiated; The radiation dose chosen must be justified in terms of its relevance to space travel; **Section E.1:** Should be changed to 36; **Section H.1:** The box for "Radioactivity" should be checked. The IACUC Chair is authorized to review and approve the response if acceptable. The final approval memo will be reviewed by the Institutional Official and will include the fact that approvals other than IACUC must be obtained before the study can begin.

I left the meeting at 1:30 pm.

IACUC Protocol

IACUC Protocol

IACUC Protocol

IACUC Meeting 01/07/10

[REDACTED]

IACUC Protocol [REDACTED]

[REDACTED]

IACUC Protocol [REDACTED]

[REDACTED]

IACUC Protocol [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

There being no further business, the meeting adjourned 2:20 pm

Respectfully submitted,

cc: [REDACTED]

BROOKHAVEN NATIONAL LABORATORY
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)
ANIMAL USE PROTOCOL



The protocol must be submitted in typed form and all applicable items must be answered. Answers must be written in English and in terms understandable to all IACUC members.

PROTOCOL #:

Title:	Ground-Based Studies in Neurobehavioral Biology	
Principal Investigator*:		
Address:	McLean Hospital, 115 Mill Street, Belmont, MA, 02478	
Phone:		
Fax:		
E-mail:		
Key Investigators*:		
* Note - if no investigators are BNL employees, please list a BNL employee contact:		
Funding Source:	NASA	BNL Account Number:
Protocol Type (e.g. Research, Teaching, Other):	Research	

A. OVERVIEW

A.1 Please provide a brief description of the proposed studies in lay terms.

The main goal of our planned studies is to examine the long-term neurobehavioral and neuropharmacological effects of space radiation in nonhuman primates. These are translational studies that are designed to forecast risk associated with human space exploration and, consequently, aid in NASA's development of future human deep space travel. The goals of these studies are based on observations that exposure to high doses of space radiation may lead to: 1) *in vitro* molecular and cellular damage in brain systems; 2) *in vitro* alterations in striatal levels of dopamine (DA); 3) short-term treatment effects in some rodent neurobehavioral assays; and 4) acceleration of the aging process with increased risk of early onset of neurodegenerative diseases. Notably, however, these findings have come from studies using *in vitro* tissue preparations or *in vivo* assays using rodents. While important first steps, NASA is interested in following this work with translationally-directed studies in primate species for confirmation and further development of its Space Radiation Program. In this work, we aim to evaluate the neurobehavioral and neuropharmacological effects of different types of ionizing radiation encountered during deep space travel. Note that this type of exposure differs from exposure to radioactive materials that may pose health risks resulting from irradiation. The health risks of space radiation at levels of exposure that are planned for these studies currently are unknown and may be trivial.

We plan to use up to 30 squirrel monkeys for the duration of the project (i.e. up to 4 years). In addition, 6 squirrel monkeys will be available as replacement subjects for these studies but will not otherwise participate in this research. In these studies, we will employ four different procedures to characterize the behavioral effects of drugs in squirrel monkeys before and after exposure to ionizing radiation. ***With the exception of the Irradiation procedure at BNL, all other procedures will be conducted at McLean Hospital.*** The four procedures include: a) observation assay of non-conditioned behavior; b) operant performance maintained by response-contingent reinforcement [e.g. food (sweetened condensed milk) delivery]; c) Stop Signal Response (SSR) Task; and d) Stimulus Discrimination/Reversal (SD/R) Task. In additional experiments, we will measure changes in the lens of the eye following exposure to ionizing radiation. In our research plan, we aim to examine the long-term neurobehavioral and neuro-pharmacological effects of exposure to two relatively low doses of ^{56}Fe (0.1 and 0.5 Gy), protons (0.5 and 1.0 Gy), and ^{28}Si (0.1 and 0.5 Gy) in squirrel monkeys. Using a within-subjects design, data will be generated in all monkeys before and after exposure to each type of space radiation. A group of 4 squirrel monkeys will be used for each radiation type and 6 squirrel monkeys will serve as controls without radiation exposure. Thus a total of 30 squirrel monkeys (6 radiation types x 4 monkeys = 24 monkeys + 6 control monkeys = 30 monkeys) will serve as subjects in these studies. Additionally, a group of 6 monkeys from our existing colony will be available as replacement subjects for these studies.

B. PERSONNEL AND TRAINING

B.1 In each box, list all personnel working directly with animals and indicate number of years of experience for each procedure for each species. All BNL personnel will be put on the appropriate Occupational Medicine Protocol. Non-BNL employees working with primates will be put on the appropriate Occupational Medicine Protocol.

NAME	SPECIES	MONITORING & HANDLING	NONSURGICAL MANIPULATION	ANESTHESIA, SURGERY	BLOOD COLLECTION	EUTHANASIA	
[REDACTED]	Squirrel Monkeys	>25 Years	>25 Years	>25 Years	N/A	>25 Years	
[REDACTED]	Squirrel Monkeys	>5 Years	>5 Years	>5 Years	>5 Years	>5 Years	
Research Assistant 1	Squirrel Monkeys	>1 Year	>1 Year	>1 Year	N/A	>1 Year	
Research Assistant 2	Squirrel Monkeys	>1 Year	>1 Year	>1 Year	N/A	>1 Year	

Note: Any personnel with less than one year experience in any of the above categories must take the applicable training listed below.

B.2 Indicate which training courses apply to this protocol. Use A to indicate all personnel or put initials of those required to take the training. All courses are located at <http://www.bnl.gov/training>

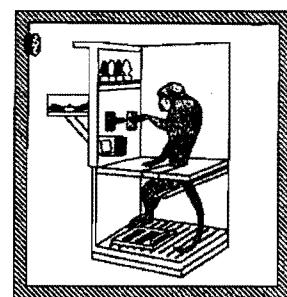
Required	COURSE TITLE	PROCEDURES COVERED
A	Basic Overview of Laboratory Animal Care and Use	Overview required by all animal users
	Biomethodology of the Mouse	Restraint, handling, identification, sexing, husbandry, behavior of mice
	Biomethodology of the Rat	Restraint, handling, identification, sexing, husbandry, behavior of rats
	Experimental Techniques in Rodents	Injections, blood sampling, oral gavage, euthanasia
	Post-Procedure Care of Mice and Rats: Reducing Pain and Distress	Analgesia, pain & distress recognition and alleviation, post-operative care
	Survival Surgery in Rodents	Anesthesia, aseptic surgical techniques
A	Primate Safety	Covers safe handling of non-human primates
	Controlled Substance Awareness and DEA background Check	Required if any controlled substances will be used
	Regulated Medical Waste Management	Required if regulated medical waste (animal carcasses, needles, syringes) will be generated as a result of the work

C. PROCEDURES

C.1 Concisely describe all manipulations and experimental procedures, including surgeries, performed on the animals. Everything done to the live animal at BNL must be detailed here. A short description of experimental procedures done elsewhere should be included. Include the end point of the experiment and timing of euthanasia, if applicable. Flow diagrams or charts are helpful. Materials and methods portion of grant applications or other detailed descriptions may be attached.

Subjects. Experimentally naïve adult male squirrel monkeys (*Saimiri sciureus*, approx. 650 to 800g) will serve as subjects (n=4/radiation type; n=6 for control group). Monkeys will be individually housed in stainless steel cages in a climate-controlled vivarium under an automated 12:12 light-dark cycle. Monkeys will have unlimited access to water and will receive a daily allotment of high-protein monkey chow (LabDiet, Brentwood, MA). All monkeys will be weighed daily and their diets will be adjusted to maintain constant body weights. Experimental sessions will be conducted daily (Monday–Friday) between 10 AM and 6 PM. The study protocols have been approved by the McLean Hospital (McL) Institutional Animal Care and Use Committee.

Apparatus. All experimental sessions will be conducted in a specifically constructed ventilated, sound-attenuating chamber provided with white noise to mask extraneous sounds. During all experimental sessions, monkeys will be seated in a customized Lexan chair. The front panel of this chair will differ for each type of experiment depending on the study requirements (see Figure). Please note that all the below procedures will be conducted at McLean Hospital with the exception of the irradiation procedure.



- a. Overt Behavior. The front wall of the chair will be removed in order to facilitate videotaping with a compact video camera (JVC, GR-AX10). The camera will be located at a distance of approximately 2ft in front of the seated monkey. All behaviors will be scored from videotape by blinded observers.
- b. PR Performance. Monkeys will be seated in a chair similar to that shown in the Figure. While seated, monkeys will face a panel containing colored stimulus lights serving as visual stimuli and two response levers. Reinforced lever presses will activate a syringe pump outside the chamber and deliver 0.2 ml of 30% sweetened condensed milk into a food tray located midway between the two levers.
- c. SSR and SD/R Tasks. During experimental sessions, the front panel will incorporate a touch screen (30cm wide x 22.5 cm high, Elo systems CarrolTouch, Menlo Park, CA) that uses an infrared sensor grid just above the surface to monitor touches. Milk will be used to reinforce behavior and will be delivered by syringe pump into a food tray located on the side wall. E-prime software (Psychology Software Tools, Pittsburgh, PA) will be used for all schedules of stimulus presentation and recording of responses.
- d. Non-Invasive QLS Assessment of the Lens. During experimental sessions, monkeys will be seated in a chair similar to that shown in the Figure. While seated, monkeys will face the Slit-lamp Stereophotonmicroscope and images of the lens of the eye will be generated.
- e. Irradiation Chamber. During irradiation procedures, freely moving monkeys will be placed individually in a customized, well-ventilated plastic chamber (25 x 25 x 25 cm) that will be positioned inside the radiation field. The chamber will be constructed to meet the requirements of the radiation field.

Transportation and Irradiation Procedure. After pre-radiation studies are completed, monkeys will be transported to NSRL/BNL for irradiation. We anticipate five separate trips to BNL (six monkey's per trip). Prior to each trip, the attending veterinarian at McL (██████████) will conduct pre-transport evaluations of all subjects, including physicals and laboratory tests to establish their pre-radiation clinical profiles, and issue required health certificates. A McL-approved vendor (Frames Animal Transportation, Ridley Park, PA) will transport monkeys to BNL. In addition to customized travel crates designed for squirrel monkeys and safe passage, food and water will be provided to monkeys *ad libitum* throughout the shipping process. Upon arrival at BNL, monkeys will be transferred to the BNL animal facility under the oversight of █████. All subjects will be individually housed in our standard stainless steel cages (Draper, Holbrook, MA). We will provide the BNL animal facility with two racks of 6 cages each; one for housing and one for cage washing. Subjects will be allowed to acclimate in the BNL animal facility for at least one week prior to radiation exposure. During that time, they will be under the direct care of BNL staff, headed by █████ (Animal Facility Manager) and █████ (Attending Veterinarian). Food, enrichment, and all instructions for the proper care of squirrel monkeys will be provided by our laboratory. For ease of handling, monkeys will wear collars and can be leashed when necessary. All irradiations will be performed in accord with BNL safety policies and procedures, and guided by BNL scientists. All subjects will be individually transferred to NSRL from the BNL animal facility in our standard transfer cages (10" x 10" x 12" high). We will provide 6 transfer cages for transporting monkeys to and from the BNL animal facility and NSRL irradiation facility. An additional 6 transfer cages will also be provided to the NSRL irradiation facility. Monkeys will remain in these transfer cages at NSRL until they are considered non-dispersible (i.e. approximately 140 min; see below).

In the present proposal, two doses of each radiation type will be studied. Separate groups of monkeys ($n = 4$) will be exposed to two doses of protons (0.5 and 1.0 Gy) at a dose rate of 0.25 Gy/min, two doses of ^{56}Fe particles (0.1 and 0.5 Gy) at a dose rate of 0.1 Gy/min, and two doses of ^{28}Si (0.1 and 0.5 Gy) at a dose rate of 0.1 Gy/min with energy levels of 1000, 600, and 600, MeV/u, respectively; a control group ($n = 6$) will be subjected to identical experimental conditions, except that they will not be exposed to any particle radiation. A total of 30 squirrel monkeys will be used for these planned experiments. The total beam time necessary to conduct irradiations in all 24 subjects plus the 6 controls will be approximately 402-min, assuming a 5-min entry and exit time from the NSRL irradiation facility per subject and a 30-min time for set-up and dosimetry per 6 monkeys (i.e. 5 trips x 30 min = 150 min); beam time calculations are based on the above described doses of each particle and its associated dose rate/min. The radiation beams and doses have been selected after consultation with NASA and BNL scientists and will provide information needed to fill critical gaps in NASA's deep space travel data base. The larger radiation field of $60 \times 60 \text{ cm}^2$ field will be used for our studies. Briefly, monkeys will be placed individually in a custom made plastic chamber that will be positioned securely and comfortably inside the radiation field perpendicular to the beam. Freely moving

monkeys will receive full body exposure to the different types of particle radiation described above. This is intended to realistically simulate the space radiation environment experienced by astronauts during deep space travel. To accurately calculate the radiation dose absorbed by an internal organ/tissue the position of all subjects will be photographed or video taped during the irradiation procedure. Following irradiation, subjects will be re-leashed and returned to the BNL Animal Facility.

We note that the activation decay times for the highest dose of protons, ^{56}Fe , and ^{28}Si particles to be considered non-dispersible are approximately 140 min, 36 min, and 30 min, respectively (see BNL website: <http://www.bnl.gov> for further details on activation decay times). Materials exposed to radiation in the BNL target room will be considered radioactive until surveyed and released by a BNL Radiological Controls Technician. Monkeys will be considered non-dispersible and will require no special radiological handling, after the de-activation time has elapsed following exposure (i.e. after approximately 140 min). Appropriate biological handling techniques including radiological training (Radworker and Radiobiology Users Training) and a radiation work permit will be obtained from BNL by [REDACTED] and trained technicians as needed. All experimental procedures at BNL and at McL will be carried out by [REDACTED] and trained technicians. Following radiation exposure, monkeys will remain at BNL for a period of at least one week. During this time, all monkeys will be monitored by BNL veterinary staff, who will issue health certificates for their return to McL. [REDACTED] will again be present during the transfer of monkeys back to McL, where the attending veterinarian will oversee the re-introduction of monkeys in the vivarium. Once fully re-situated, monkeys will be tested periodically and on a long-term basis, i.e., up to 3–4 years following exposure to particle radiation.

Experimental End Point and Euthanasia. Upon completion of all studies, subjects will remain in the McL colony under the direct care of the McL staff and the attending veterinarian at McL. No further studies are planned for these subjects. Euthanasia is not an end point in these experiments.

C.2 Does this work duplicate previous experiments/activities? If yes, justify.

No. To our knowledge, long-term effects of ionizing radiation on the CNS functioning of nonhuman primates have not been examined previously.

D. ANIMAL DESCRIPTION

D.1 Species:	Squirrel Monkeys
D.2 Strain/Breed:	Saimiri sciureus
D.3 Sex:	Male
D.4 Age/Weight:	Adult / Weight = 650 – 800g
D.5 Supplier:	N/A

If not a commercial vendor, a recent health report (no older than three months) from the animal facility must be submitted to the BLAF Manager at least six weeks before the planned experiment or shipment of animals. Please contact the BLAF Manager at [REDACTED] to make arrangements for the receipt of the animals.

Health reports for all animals will be submitted to the BLAF Manager 6 weeks prior to planned shipment/experiment. Health reports for each monkey will be generated by the attending veterinarian at McL ([REDACTED]).

D.6 Justify that the work is appropriate to be done in an animal model.

The proposed research is not ethically permissible in human subjects. The work also cannot be conducted in rodent or other lesser species because of the long-term nature of these studies, the use of procedures that are not feasible across species, documented differences in the neurobiological actions of many drugs across species, and the unique translational value of data obtained in nonhuman primates. The research also cannot be conducted using tissue samples or other biological material or computer modeling because the goal of the research is to understand the long-term effects of ionizing radiation on CNS processes in nonhuman primates under conditions predictive of effects in humans. See section D7 for further details.

D.7 Justify species to be used and why a lower phylogenetic species cannot be used.

Under the experimental conditions described in this application, the effects of ionizing radiation-induced changes in the behavioral pharmacology of nonhuman primates can be associated meaningfully to their effects in humans. Squirrel monkeys have been selected for the proposed studies because several features of nonhuman primate research facilitate the translation of experimental results to humans with reasonable predictive validity.¹⁻⁶ For example, a) nonhuman primates and humans are similar in their genetic, physiological, pharmacokinetic, and neurobiological characteristics; b) they have been studied previously under experimental conditions similar to those detailed in this application; c) an extensive literature regarding the physiological and pharmacological effects of drugs in this species is available for reference; d) nonhuman primates are reliable subjects in long-term behavioral and pharmacological studies¹⁻⁶; e) they adapt well to laboratory environment and can be handled easily and safely by experienced staff members; and f) within-subject designs similar to those used in human laboratory studies permit meaningful conclusions or inferences to be based on the evaluation of all treatment effects in individuals as well as in groups. Together, this information allows us to conduct our research efficiently and is fundamental to a constructive assessment and interpretation of data. All these considerations suggest that nonhuman primates are especially well-suited for ground-based research to study long-term neurobehavioral effects of space radiation. To date, the effects of space radiation have mainly been examined in *in vitro* preparations or neurobehavioral assays in rodents. These studies provide a strong foundation for further assessing the immediate and long-term CNS effects of space radiation. Cognizant of anatomical and functional variations across species that limit extrapolation of laboratory data from rodents to nonhuman primates or humans, our approach will employ well-established procedures in nonhuman primates to study the effects of ionizing radiation on brain systems that may be involved in CNS processes.

D.8 Animal Numbers

D.8.a Total for first three years:	Total: 36 Squirrel Monkeys 24 monkeys exposed to particle radiation; 6 monkeys as controls; 6 as replacement subjects
D.8.b Maximum housed at one time:	6 Squirrel Monkeys

D.9 Justify number of animals. Indicate design of study groups and statistical methods and include power calculations. Include steps taken to minimize the number of animals required.

In the present proposal, two doses of each radiation type will be studied. Separate groups of monkeys ($n = 4$) will be exposed to two doses of protons (0.5 and 1.0 Gy), ^{56}Fe particles (0.1 and 0.5 Gy), and ^{28}Si (0.1 and 0.5 Gy); a control group ($n = 6$) will be subjected to identical experimental conditions, except that they will not be exposed to any particle radiation. A total of 30 squirrel monkeys will be used for these planned experiments. Additionally, 6 squirrel monkeys will be available as replacement subjects for these studies. We recognize that non-human primates are a valuable resource, and our research is designed to minimize the number of subjects. The within-subject design used in the proposed research, in which each animal serves as its own control, allows for scientifically meaningful results to be obtained with fewer animals (usually four subjects per experiment) that would be necessary with other experimental designs.

Data Analysis. Please note that all procedures described below will be conducted at McLean Hospital. In observational studies, the effects of vehicle and each drug dose will be determined by calculating the average group frequency or duration of target behaviors during 5-min observation periods. For cumulative dosing procedures, data from the test component immediately following injection will be used to express the effects of the administered dose, and for single dosing procedures, data from all four test components will be averaged and used to express the effects of the administered dose. For operant performance under a progressive ratio schedule, i.e., PR responding, the measures for each monkey will be: a) the value of the final ratio completed for milk delivery, i.e., the break point; b) the total number of lever presses in each component; and c) the mean response rate. In the SSR Task, impulsivity will be measured by the stop signal response time (SSRT) determined for each session (SSRT = mean Go reaction time – mean SSD). Performance monitoring will be the Post–Stop trial Slowing (PSS) determined as: mean of the individual values of: PSS = Go reaction time of the first Go trial following a Stop trial – Go reaction time of the last Go trial prior to that Stop trial. Data for the SD/R Task will be expressed as the number of stimulus discriminations divided by the total number of trials and the number of trials to criterion following stimulus reversal.

Non-Invasive QLS Assessment of the Lens. Primary outcome is the total light scattering intensity and autocorrelation function parameters (continuous variable). All results will be presented as group means (\pm S.E.M.). Where possible, data will be analyzed using analysis of variance followed by post hoc tests for specific comparisons (significance will be set at $P < 0.05$). Dose-effect curves will be analyzed with standard parallel-line bioassay techniques. $^{124}\text{ED}_{50}$ values and their 95% confidence limits will be determined and pairs of ED_{50} values will be considered to be significantly different if their 95% confidence limits do not overlap.

E. PAIN/DISTRESS

E.1 List total number of animals at applicable levels of stress/discomfort

Level A: No pain or distress: Animals will be euthanized without any treatments or manipulations or irradiation with unrestricted movement and without anesthesia and without anticipated subsequent effects at BNL.

Level B: Relived or momentary pain or distress: Momentary pain or potential pain or distress relieved by pharmacologic, behavioral or other means, e.g., injection of any substance including anesthetics, post-procedural analgesics, behavioral conditioning, restraint or minor pain/distress and medical treatment of disease states.

Level C: Unrelieved or sustained pain or distress: Any procedure that would cause more than momentary or slight pain or distress, e.g., chronic untreated disease states, pain research

Species	LEVEL A	LEVEL B	LEVEL C
Saimiri Sciureus – Squirrel Monkeys		30	

E.2 For animals used in Level B or C, perform a literature search for alternatives to pain/distress.
Please note the Research Library Staff is available to assist with literature searches.

List procedures that may cause pain/distress (e.g. irradiation, imaging, surgery, injection, behavioral testing, food restriction, etc) and perform a search using the procedures and the word "alternative". Procedures that have pain eliminated by the use of anesthetics and/or analgesics are still considered painful even though the animal is not expected to experience any pain/distress.

Irradiation Procedure

Date of Search:	10/28/09
Databases Searched:	PubMed
Years included:	1960 – 2009

No suitable alternative methods exist

Provide a narrative of Search Results When alternative procedures are discovered, you must identify them and justify why those procedures are not being considered:

We typically conduct searches on a bi-annual basis with key terms of squirrel monkey, cognitive tasks, motivation, schedule-controlled behavior, alternative methods. We have incorporated the key term of space radiation in our most recent search (10/28/09), and will continue to do so.

E.3 Indicate how procedures have been refined to reduce the amount of potential pain, distress or morbidity.

Laboratory procedures for our planned studies do not involve pain and have been developed over decades to minimize distress to experimental subjects. Briefly, animals are trained by experienced laboratory personnel to perform behavioral tasks while loosely restrained at the waist within customized primate chairs placed within larger ventilated enclosures. Outside of experimental sessions, lab staff members supervise and interact with animals for which they are responsible, providing them with enrichment and care.

E.4 Describe if animals are subjected to food/water deprivation or prolonged and/or unusual restraint and provide justification. Describe how animal health is monitored during deprivation.

All animals will have free access to food and water and will not be subjected to prolonged or unusual restraint.

E.5 Is death used as a study endpoint wherein animals must die without intervention such as pain relief and/or euthanasia? If yes, explain why an earlier end point is not acceptable.

No

F. ANIMAL CARE

F.1 Please indicate if animals will be housed at BNL in other than in the Brookhaven Laboratory Animal Facility (BLAF). All singly-housed rodents will be provided with environmental enrichment unless scientifically justified.

No

F.2 Describe additional requirements for other than routine animal care (e.g. housing, feeding)
Investigative staff must be responsible for feeding all animals, weighing the correct amount of food, logging each feeding and adjusting the ration as needed to maintain the animal at the desired weight. If food, equipment and/or other supplies are to be shipped from another institution's animal facility, a recent health report from the facility must be submitted to the BLAF Manager at least six weeks before the planned experiment.

Food (LabDiet, Brentwood, MA), equipment (i.e. housing cages), and all other supplies will be shipped directly from the vendor to BLAF at least six weeks prior to the planned shipment/experiment. Instructions on housing, feeding, and all other routine animal care will be provided to the BLAF Manager by our laboratory.

F.3 List the building and room number(s) in which experimental procedures, surgery, and/or postoperative recovery will be performed on live animals (if known).

With the exception of exposing monkeys to space radiation at NSRL, no other experimental procedures are being conducted at BNL.

G. PROCEDURE SPECIFICS

G.1 List all chemical agents (sedatives, analgesics, anesthetics, paralytics, euthanasia, study drugs, radiotracers) administered to the animals. For euthanasia involving CO₂, please use 100% CO₂ at a 20% air replacement per minute rate. For ketamine anesthesia, please use intraperitoneal (ip) injections, not intramuscular (im). Ketamine/xylazine may be stored for up to 28 days after mixture.

Type	Agent	Dose	Route	Frequency	Controlled Substance (Y/N)
N/A					

G.1.a List the name(s) of the individual(s) administering the above agents:

N/A

G.1.b Indicate building and room numbers where agents are stored and security procedures for controlled substance(s):

N/A

G.1.c If paralytic agents are used in conjunction with surgical manipulations, indicate the means by which absence of pain is monitored and/or determined, and who is responsible:

N/A

G.2 Is surgery involved? If yes, indicate whether surgery is survival or non-survival.

N/A	G.2.a Describe monitoring and supportive care provided during surgery (who, what and how will this be done?):
N/A	G.2.b Describe indications for analgesic therapy to be administered before, during, and/or following surgery:
N/A	G.2.c Describe post-operative and/or anesthetic monitoring and supportive care (who, what and how often): Please use Surgery and Recovery Record
N/A	G.2.d Who will maintain surgical and post-operative records and where will they be maintained? Please note: Records must be accessible for inspection
N/A	G.3 Is anesthesia involved?
N/A	G.3.a Describe monitoring and supportive care provided during anesthesia (who, what, and how will this be done?): Please use Surgery and Recovery Record
N/A	G.3.b Who will maintain anesthetic records and where will they maintained? Please note: Records must be accessible for inspection
N/A	G.4 Are animals to be used in more than one major surgical procedure from which they are allowed to recover? If yes, please describe and justify.
N/A	G.5 By what method and by whom will animals be euthanized and how will death be confirmed? If a chemical agent is used, please list in Section G.1. For euthanasia involving CO₂, please use 100% CO₂ at a 20% air replacement per minute rate. Justification must be provided for any physical method, such as decapitation or cervical dislocation, without anesthesia.
If and when recommended by veterinary staff, monkeys will be euthanised by i.v. pentobarbital (100mg/kg) followed by thoracotomy. McLean veterinarian [REDACTED] (>15 yrs experience) will conduct all euthanasia procedures.	

G.6 List criteria for intervention and/or removal of animals from study or early euthanasia.

• Examples are severe ataxia; rapidly increased heartrate or respiratory rates; oral, nasal or vaginal discharge such as pus or blood; wound dehiscence; marked swelling, tumor(s) greater than 2 cm or ulcerating, ulcer greater than 10% of body surface area, inability to eat or drink, loss of weight, great discoloration in an appendage or surgical area; immobility.

• Unless otherwise noted 100% CO₂ at a 20% air replacement per minute rate will be used for early euthanasia for rodents.

Animals will be removed from study in the unlikely event of major organ system failure (e.g., heart, liver, kidney) or if there are signs of persistent behavioral distress evident in dramatic changes in home cage activities (e.g., decrease in activity and response to external stimuli or unexplainable loss of weight or appetite). Such determinations will be made in consultation with veterinary staff.

H. SPECIAL CONSIDERATIONS N/A**H.1 Check hazardous materials being used in this study.**

<input type="checkbox"/> Human cells or fluid	<input type="checkbox"/> Microorganism	<input type="checkbox"/> Chemicals including fixatives	<input type="checkbox"/> Recombinant DNA
<input type="checkbox"/> Nanoparticles	<input type="checkbox"/> Radioactivity	<input type="checkbox"/> Other (list)	<input type="checkbox"/>

For each agent listed above, please ensure that it is covered under an approved ESR

H.2 Indicate if animals will be shipped from BNL. If yes, indicate that BNL's preferred shipping procedures will be followed. If other arrangements are necessary, please describe.

Yes. Both BNL and McL shipping procedures will be followed as described in Section C1, under transportation and irradiation procedure.

H.3 If not shipped from an approved vendor, detail how animals will be transported to BNL.

Animals will be transported to and from BNL and McL as described in Section C1, under transportation and irradiation procedure.

I. INVESTIGATOR ASSURANCE

I affirm to the best of my knowledge that all the above information is complete and accurate and agree to accept responsibility for this project in accordance with applicable Federal and State of New York regulations, USDA guidelines, and established BLAF policies and procedures. No changes will be made without prior approval from the IACUC.

In order to reduce risk to all personnel and laboratory animals, I agree to:

- a. Follow BNL procedures for aspects of the animal care and use such as preoperative care, anesthesia, surgical technique, postoperative care, sampling techniques, euthanasia, and disposal of contaminated carcasses and waste.
- b. Ensure that my instructions to laboratory personnel are implemented.
- c. Ensure that all project personnel comply with the required Occupational Health Program before handling animals.
- d. Instruct all personnel in my laboratory that they should inform me if they believe that the treatment of any research animal is inappropriate. If the situation is not resolved, the employee should contact the Attending Veterinarian, or the IACUC Chair and/or Institutional Official.

I am aware that all research outlined under this protocol must be carried out under approved Experimental Safety Review(s) (ESR). I am aware that it is my responsibility to ensure that all individuals working on this protocol have been listed on an appropriate ESR and that their training is up to date. I am aware that work cannot proceed without an approved ESR.

PRINCIPAL INVESTIGATOR	[REDACTED]	DATE	12/22/09
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Your Department Safety Coordinator will be notified of your IACUC approval.

J. APPROVALS

I attest that the following issues have been appropriately addressed: Scientific merit of project; Appropriateness of conducting the project at BNL; Adequacy of funding for the project; Appropriateness of the expertise and experience of the PI and project personnel; Appropriateness of training for the PI and project personnel, and; Adequacy of department resources to support this protocol.

BNL DEPARTMENT CHAIR		DATE	
PHARMACIST (or designee)		DATE	

Required for Schedule I controlled substances

References:

- 1 Weerts EM, Fantegrossi WE, Goodwin AK (2007) The value of nonhuman primates in drug abuse research. *Experimental Clinical Psychopharmacology*. 15:309-327.
- 2 Goldman-Rakic PS, Lidow MS, Smiley JF, Williams MS (1992) The anatomy of dopamine in monkey and human prefrontal cortex. *Journal of Neural Transmission*. 36: 163-177.
- 3 Ward KW, Smith BR (2004) A comprehensive quantitative and qualitative evaluation of extrapolation of intravenous pharmacokinetic parameters from rat, dog, and monkey to humans. I. Clearance. *Drug Metabolism and Disposition*. 32:603-611.
- 4 Matta SG, Balfour DJ, Benowitz NL, Boyd RT, Buccafusco JJ, Caggiula AR, Craig CR, Collins AC, Damaj MI, Donny EC, Gardiner PS, Grady SR, Heberlein U, Leonard SS, Levin ED, Lukas RJ, Markou A, Marks MJ, McCallum SE, Parameswaran N, Perkins KA, Picciotto MR, Quik M, Rose JE, Rothenfluh A, Schafer WR, Stoleman IP, Tyndale RF, Wehner JM, Zirger JM (2007) Guidelines on nicotine dose selection for in vivo research. *Psychopharmacology (Berl)*. 190:269-319.
- 5 Czoty PW, Makriyannis A, Bergman J (2004) Methamphetamine discrimination and in vivo microdialysis in squirrel monkeys. *Psychopharmacology (Berl)*. 175:170-178.
- 6 Mutschler NH, Bergman J (2002) Effects of chronic administration of the D₁ receptor partial agonist SKF 77434 on cocaine self-administration in rhesus monkeys. *Psychopharmacology*. 160:362-370.