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

The protocol must be typed and all questions must be answered. Answers must be written in English and in lay terms understandable to all IACUC members.

PROTOCOL #	517

Title	PROBING THE SYNERGISTIC EFFECTS OF RADIATION, ALTERED GRAVITY AND STRESS ON BEHAVIORAL, COGNITIVE AND SENSORIMOTOR FUNCTIONS TO PREDICT PERFORMANCE DECREMENT IN ASTRONAUTS		
Principal Investigator*	Susanna Rosi		
Institution	University of California San Francisco		
Street Address	1001 Potrero Ave., San Francisco General Hospital, Building#,1 Room 242, San Francisco, CA 94110		
Phone	415 206 3708		
Fax	415 206 3948		
E-mail	Susanna.rosi@ucsf.edu		
Key Investigators*	Susanna Rosi, Andrew Wyrobek, Tami Jones, Vivian Mao, Ana Mora		
** Note - if no investigators are BNL employees, please list a BNL employee contact*	Peter Guida		
Funding Source ***Submit animal methods section of grant***	NASA NNX14AC94G		
Protocol Type (e.g. Research, Teaching, Other)	IACUC Research		
Home Institution IACUC Approval # and dates	AN181839-01 12/2/19-12/2/2022		

A. OVERVIEW

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

Ex. 4 - Business confidential/ Proprietary info - experiment details

The objective of the proposed study is to understand the effects of low doses of galactic cosmic (GCR) irradiation, simulated microgravity [redacted] and social isolation on cognitive functions in rodents. Furthermore, we will look at the changes in the cellular and molecular endpoints important for the maintenance of proper cognition. These changes are dynamic and require input from multiple cell types inside and outside of the hippocampal formation. [redacted]. Oxidative stress and inflammation play a significant role in cognitive impairments following low doses of space irradiation. [redacted] The biochemical/molecular pathways identified in this proposal will provide potential targets for protections against these space stressors or intervention to prevent cognitive impairments in space crews during and after space missions.

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.

NAME	SPECIES	MONITORING & HANDLING	NONSURGICAL MANIPULATION	ANESTHESIA, SURGERY	BLOOD COLLECTION	EUTHANASIA
Gregory Nelson, PhD	mice	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Tami Jones, BS	mice	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Mary Campbell-Beachler, BA	mice	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Susanna Rosi, PhD	mice	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Vivian Mao, PhD	mice	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Katherine Grue, MS	mice	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Exs. 4/6 Business confidential/ Proprietary info relating to experiment details/ Personal information

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**
A	**Regulated Medical Waste Management**	**Required if regulated medical waste (animal carcasses, needles, syringes) will be generated as a result of the work**
The courses below are for BNL employees or guest researchers who have less than one year of training. Guest researchers must have appropriate training from their home institution.		
	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
	Anesthesia in Laboratory Animals	Anesthesia techniques
	Survival Surgery in Rodents	Aseptic surgical techniques

C. PROCEDURES

C.1 Concisely describe all manipulations and experimental procedures performed on animals.
Everything done to the live animal at BNL must be detailed here, including the timing of shipment to/from BNL. A short description of experimental procedures done at collaborating institutions (if applicable) should be included. Include the end point of the experiment and timing of euthanasia, if applicable. Flow diagrams or charts are helpful.

[redacted]

[redacted]

[redacted]

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

No

D. ANIMAL DESCRIPTION

D.1 Species	Mus Musculus. Strain C57BL/6J
D.2 Strain/Breed	Mus Musculus. Strain C57BL/6J
D.3 Sex	Male and Female
D.4 Supplier	Jackson

*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 two weeks before** the planned experiment or shipment of animals. Please contact the BLAF Manager at (631) 344-3620 to make arrangements for the receipt of the animals. Rodents less than three weeks old must be received with their dams.*

D.6 Justify that the work is appropriate to be done in an animal model. *Indicate why a computer or non-animal model is not a viable alternative.*

Our goal is to study cognitive function and how combined space stressors could modify or affect cognitive function. To this extent we need animal model to study basic fundamental function that cannot be recapitulated by computer modeling or non-animal model yet.

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

Laboratory mice are the smallest mammalian species used in research with well-characterized genetic background and are relatively easy for genetic manipulation. Inbred and F1 hybrid mice with specific genetic alterations provide consistent phenotype independent of when the mice are generated and which breeder pairs are used. Therefore, one can sample the population repeatedly for mechanistic studies. Additionally, mice can be produced in large numbers in a relatively short time, and do not require extensive housing space, making it an ideal model for in vivo studies. [redacted]

Ex. 4- BUsiness confidential/ Proprietary info experiment

D.8 List total number of animals to be used.

[redacted]

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. Flow diagrams or charts are helpful.

[redacted]

Ex. 4 - Business confidential/ Proprietary info experi-details

[redacted]

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 animals will undergo irradiation with minimally restricted movement and no anesthesia and without anticipated subsequent effects while at BNL.

Level B: Relieved 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
Mus Musculus	[redacted] Ex. 4 - Business Confidential/Proprietary info		[redacted] Ex. 4 - Business Confidential/ Proprietary info experiment details

Include scientific justification for any animals in Level C.

[redacted]

E.2 For animals used in Level B or C, perform a literature search for alternatives to pain/distress.

Use this link for search tips: https://www.nal.usda.gov/awic/awic-tips-searching-alternatives-animal-research-and-testing

List procedures that may cause pain/distress (e.g. imaging, surgery, injection, behavioral testing, food restriction, etc) and perform a search using the procedure terms. *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.*

[redacted]

Ex. 4 - Business Confidential/Proprietary info experiment details

Date of Search	02/05/20
Databases Searched	Pubmed
Key Words Searched. *Include the word "alternatives"*	[redacted] Ex. 4 - Business Confidential/ Proprietary info experiment details

Provide a narrative of Search Results. *List how many "hits" were obtained for key words used. If alternative procedures are discovered, identify them and justify why those procedures are not being considered*:

[redacted] Ex. 4 - Business confidential/ Proprietary info experiment details

E.3 For animals used in Level B or C, indicate how procedures have been refined to reduce the amount of potential pain, distress or morbidity.

We have reduced the n to the smallest number of animals required to undergo [redacted]. The animals will be observed daily by investigators to monitor changes in body weight, coat condition and grooming behavior, eye and nasal discharge, dehydration, body position and movement will all be used to determine pain or distress in the animals.

Ex. 4 - Business Confidential/ Proprietary info experiment details

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/restraint.*

[redacted]. Ex. 4 - Business confidential/ Proprietary info experiment details

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.*

NA

F. ANIMAL CARE

F.1 Describe additional requirements for other than routine animal care (e.g. housing, feeding, hazardous waste bedding disposal).

[redacted] Ex. 4 - Business confidential/Proprietary info experiment details

F.2 Scientifically justify if animals must be singly-housed (excluding male mice).

[redacted]

The rationale for these stressors is described in section C1. Ex. 4 - Business Confidential/ Proprietary info experiment details

F.3 Scientifically justify if animals will not be provided with environmental enrichment.
NA

G. PROCEDURE SPECIFICS

G.1 List all chemical agents (Sedatives, analgesics, anesthetics, paralytics, euthanasia, study drugs, radiotracers) administered to the animals. *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) *
NA					

*Note: Controlled Substance Awareness training and a DEA background Check will be required for anyone using controlled substances.

For any non-pharmaceutical grade drugs/substances, the preparation and use of the drug must be scientifically necessary, appropriately justified and approved by the IACUC, taking into consideration the side effects, stability, sterility and aseptic handling, storage requirements and other considerations associated with the preparation of this agent. *Please provide information in the box below for all non-pharmaceutical grade substances listed above.*

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

G.2 Is surgery involved? *If yes, indicate whether surgery is survival or non-survival.*
NA
G.2.a Describe monitoring and supportive care provided during surgery *(who, what and how will this be done?)***:**
G.2.b Describe indications for analgesic therapy to be administered before, during, and/or following surgery.

G.2.c Describe post-operative and supportive care (*who, what and how often).* ***Please use Surgery and Recovery Record (link to document)***

G.2.d Who will maintain surgical and post-operative records and where will they be maintained? *Records must be accessible for inspection and originals for USDA covered species must remain at BNL.*

G.3 Is anesthesia involved?

NA

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 (link to document)***

G.3.b Who will maintain anesthetic records and where will they maintained? *Records must be accessible for inspection and originals for USDA covered species must remain at BNL.*

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.*

NA

G.5 For euthanasia performed at BNL (planned or unplanned), what method and by whom will animals be euthanized and how will death be confirmed? If a chemical agent is used, please list it in Section G.1. *For euthanasia involving CO_2, please use 100% CO_2 at a 60% air replacement per minute rate. Justification must be provided for any physical method, such as decapitation, without anesthesia.*

If they show signs of discomfort due to traveling or aggression in the cage or any other unforeseen reason, we will use 100% CO_2 at a 60% replacement rate. Additionally, investigators will confirm death with either a cervical dislocation or decapitation. [redacted] euthanasia at BNL.

Exs. 4/6 - Business confidential/ Proprietary info/ Personal experience

G.6 List criteria for intervention and/or removal of animals from study or unplanned 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, inability to eat or drink or loss of weight greater than 15%.*
- *Unless otherwise noted, 100% CO_2 at a 60% air replacement per minute rate will be used for early euthanasia for rodents.*

Ex. 4 - Business confidential/ Proprietary info experiment details

We will monitor the mice daily at the start of the stressor ([redacted]) and following irradiation to identify potential illnesses. In the unlikely situation where any mouse appears to be in distress, we will consult with the veterinary staff and the mouse will be euthanized if it meets the euthanasia criteria: 15% change in body weight; tumor size larger than 1 cm in diameter; non-resolving

skin lesions; obvious signs of anemia, dehydration, weakness, or motor problems.

H. SPECIAL CONSIDERATIONS

H.1 Check materials used in this study that are hazardous to personnel.

☐ **Human cells or fluid**	☐ **Microorganism/Virus**	☐ **Chemicals including fixatives**	☐ **Recombinant DNA**
☐ **Nanoparticles**	☐ **Radioactivity (isotopes)**	☒ **Irradiation**	☐ **Other (Specify)**

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, we will use BNL shipping procedures to send the animals back to UCSF

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

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 BNL policies and procedures.

No changes will be implemented without 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 project personnel are implemented.

c. Ensure that all project personnel complete all required training 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, the IACUC Chair and/or Institutional Official.

e. Ensure that all research outlined under this protocol shall be carried out under approved Experimental Safety Review(s) (ESR).

PRINCIPAL INVESTIGATOR	[redacted]	**DATE**	Ex. 6 - Personal info - signature 03/06/20
Your Department Safety Coordinator will be notified of your IACUC approval.			
BNL DEPARTMENT		**DATE**	