Topical: Development of a NASA Space Stressors Laboratory (NSSL) Leveraging Existing Resources

Authors: Jason M. Weeks[1], Janice Zawaski[1], Janapriya Saha[2], Brock J. Sishc[3], and S. Robin Elgart[4]

Primary Author Information:
Jason M. Weeks
832-248-6508
Nation Aeronautics and Space Administration (NASA)
jason.weeks-1@nasa.gov

Affiliations:

1. Nation Aeronautics and Space Administration
   Johnson Space Center
   Houston, TX
2. Leidos
   Reston, VA
3. Aegis Aerospace, Inc.
   Houston, TX
4. University of Houston
   Houston, TX

Topical: Development of a NASA Space Stressors Laboratory Leveraging Existing Resources

As the National Aeronautics and Space Administration (NASA) prepares for future missions to the Moon and then on to Mars, many of the health risks of cosmic radiation still need to be characterized and mitigated. To support these goals, NASA partnered with the Office of Science for the U.S. Department of Energy (DOE) to establish the NASA Space Radiation Laboratory (NSRL) at DOE's Brookhaven National Laboratory (Figure 1). This facility allows scientists to use high energy ion beams to simulate space radiation and assess the risks of space radiation on biological outcomes and equipment[1]. **To capitalize on the unique ability of NSRL to simulate the space radiation environment and leverage available state-of-the-art specimen preparation resources, an expansion to the current capabilities is recommended to facilitate and accelerate integrated research on combined space hazards and risks including altered gravity, isolation/confinement, sleep and create a NASA Space Stressors Laboratory (NSSL).**

The NSRL delivers ions that are created by the Brookhaven National Laboratory Booster accelerator then routed and focused to the NSRL facility.

Figure 1. NASA Space Radiation Laboratory (NSRL) at Brookhaven National Laboratory.

Current NSRL irradiation capabilities:

- Dose-rates of approximately 10 Gy/min using a 20 × 20 cm^2 beam area and approximately 0.5 Gy/min using a 60 × 60 cm^2 beam are available. Dose fractionation across multiple days is also possible.
- The exposure structure, referred to as "spills", for most radiobiology exposures has a four second repetition time. During this four second period, the ions are extracted uniformly in time during a 0.3-0.4 second spill, followed by a ~3.6 second dead time when the beam is off.
- Single ion or mixed-beam field irradiations are possible including GCRSim and SimGCRSim [1]. For ion and energies availability, see **Table 1**.

Schedule: The NSRL is scheduled for three runs per year; Spring (March – May), Summer (June – July), and Fall (Sept – Nov). **Cell Culture Capabilities**: Two labs for cell/tissue culture procedures are available as well as one lab for additional processing of samples including two chemical fume hoods (~325 sq. ft./room)

Vivarium: In the current configuration, approximately 8000 rodents/year can be supported for animal-based experiments. The animal husbandry system at NSRL includes five caging racks and a ventilation/filtration system in largest (250 sq. ft.) room, and two smaller rooms (175 sq. ft. each) with conventional caging systems (Figure 2).

Figure 2. NSRL's rodent vivarium.

Establishing a Space Stressor Laboratory:
Currently, the capabilities of NSRL focus on facilitating space radiation research. However, with additional infrastructure and equipment, **NSRL could be established as a NASA Space Stressors Laboratory – a one-of-a-kind facility – to provide a robust platform for spaceflight research**. The following recommendations range in complexity from adding unique infrastructure (e.g. neutron irradiation source, centrifuges for coincident microgravity research) to simple equipment purchases (in-vivo imaging). It is important to note that depending on the complexity of upgrades and additional workload, auxiliary personnel may be required to support the equipment as well as of future experiments. Furthermore, maintenance costs will need to be incorporated into budgetary planning to ensure success.

Infrastructure recommendations:

1) **Neutron exposures** – Neutrons are constituents of the space radiation environment that account for approximately 10-15% of dose inside a spacecraft due to the interaction of GCR with matter as it passes through the spacecraft hull (Norbury Citation). When charged particles interact with the surface of a moon or planet, they also create neutrons that backscatter (albedo neutrons) that astronauts will be exposed to during surface operations. Currently a neutron facility at Colorado State University, Fort Collins is used to conduct

Ion Species [1]	Max Energy [2] (MeV/n)	LET in H_2O at Max Energy (keV/micron)	Peak LET (keV/micron)	Range in H_2O (mm)	Maximum Intensity [3] (ions per spill)
H^1	2500	0.206	84.3	10490	2.2×10^{11}
He^4	1500	0.84	237	5550	0.3 x 10^10
C^{12}	1500	7.55	922	1856	1.2×10^{10}
O^{16}	1500	13.4	1306	1391	0.4×10^{10}
Ne^{20}	1000	21.9	1637	657	0.10×10^{10}
Si^{28}	1000	43.4	2519	463	0.3×10^{10}
Ar^{40}	1000	74.2	3268	387	0.02×10^{10}
Ti^{48}	1000	105.6	3924	327	0.08×10^{10}
Fe^{56}	1000	147	4706	274	0.2×10^{10}
Kr^{84}	721	314	6221	132	2.0×10^7
Nb^{93}	520	594	6690	70	1×10^7
Ag^{107}	575	576	8470	70.7	3.5×10^6
Xe^{129}	589	761	9788	68.3	5.0×10^7
Ta^{181}	475	1449	12300	39.2	5.0×10^7
Au^{197}	400	1865	13140	27.7	1×10^8
Bi^{209}	359	21.8	138.7	22.6	7.0×10^7
Sequential Field	Various	Various	Various	Various	Various
Solar Particle Event [5]	Various	Various	Various	Various	Various
GCR Simulation	Various	Various	Various	Various	Various

Table 1. Ion Species and energies available at NSRL

neutron studies at space relevant doses and dose rates. This physical separation (over 1,800 miles) poses a significant experimental design challenge and knowledge gap for combined exposures of GCR and neutrons because it will require irradiating animals at NSRL followed by transportation to Fort Collins, Colorado or vice versa. Thus, no "combined" exposures are currently possible. Lack of neutron radiation facility at NSRL hinders research to characterize the biological impacts of neutrons with heavy ions. It is recommended that an appropriate neutron source be installed within a vivarium room capable of housing rodents to improve relevance of future experiments. Since NSRL was recently upgraded to a state-of-the-art GCR simulator, adding neutron radiation capability will position it as the world's only facility to be able to simulate a near-complete radiation environment.

2) **Centrifuges and Clinostats** – With the aim of upgrading NSRL to a comprehensive space stressors laboratory, on-site clinostats will allow the interrogation of microgravity effects in combination with space radiation on plants and cell cultures. Additionally, the inclusion of centrifuges (cellular and animal) would allow for additional research on the effects of G transitions on physiology.
3) **Vivarium** – In addition to the functional rodent vivarium described above, NSRL currently has 12 mini-pigs runs that need minor repairs and updates due to age and deferred maintenance. Once repaired, the cost and time required to perform maintenance on the runs is anticipated to be minimal. Additional upgrades to the vivarium could facilitate additional research in unique model systems such as drosophila, nematodes, and fish (zebra, medaka). Note that Medaka fish have previously been utilized by Japan Aerospace Exploration Agency (JAXA) on the International Space Station. Lastly, sleep deprivation, isolation, confinement, and microgravity are concerns for long duration space missions. It is recommended that equipment within the vivarium is upgraded to allow for additional sleep-related research risk including sleep deprivation chambers, multiple cage sizes cages, isolated cages, and unloading cages.

Equipment Recommendations: The processing, read-out and analysis of the majority of space radiation research are performed weeks following irradiation at the Principal Investigator's home institution due to inadequate equipment availability at NSRL, thus limiting research outcomes to late effects, while immediate or "in-mission" effects are possibly lost when extrapolated to human space missions. The recommendations below would also allow for acute measurements of space radiation effects and potentially combined stressors if infrastructure recommendations (see above) are incorporated.

1) ***In-vivo* Imaging**: Currently, NSRL does not have any *in-vivo* imaging capabilities which limits collection of immediate measurements without euthanizing animals for tissue assessment. The following technologies are recommended to upgrade the NSRL:
   a) Ultrasound (e.g. Visual Sonics Vevo 3100, Vevo LAZR-X) would add the capability of anatomical, functional, and molecular imaging on major organs and vasculature.
   b) Micro-CT would enable high resolution bone imaging as well as visualization of gross changes in other tissue/organs.
   c) Bioluminescence/fluoroscopic imaging would allow reporter assays such as firefly luciferase and fluorescent molecular markers to quantitatively measure dynamic changes in gene expression and disease progression longitudinally.

2) **Behavioral/Cognition Equipment**: Currently no equipment resides at NSRL to capture behavioral or cognitive outcomes for rodents limiting the analysis of immediate impacts of stressor exposures that may align with in-mission effects. The integrated central nervous system, behavioral medicine, and sensorimotor (CBS) research project within the Human Research Program has recently proposed and is reviewing a standard behavior/cognition testing paradigm (Figure 3) which could be used to prioritize the purchase of specific equipment.

	Cognition - Weighted Battery	Broad Domain Battery
5 choice - Continuous performance test (preferred) or PVT (substitute)	✓ (red)	
Home Cage Monitoring		✓
Open Field Behavior	✓	✓
Elevated Plus/Zero Maze	✓	✓
Forced Swim/Tail Suspension with Sucrose Preference Test		✓
Balance Beam		✓
Adhesive Removal Test	✓	✓
Radial Arm Maze (+/- water)		✓
Odor Span Test	✓ (red)	✓
3-Chamber Social Interaction Test		✓

Figure 3. Proposed standardization of cognition/behavioral testing for future funded studies. Cognitive/Behavioral tests were selected based on their brain domains and equivalency to human tasks. Due to the number of proposed tests and the extensive training required (red arrow) the tests were divided into two batteries: cognition-weighted battery and broad domain battery that would utilize unique cohort of animals.

3) **Tissue on a Chip (TOC):** NASA is currently funding several experiments utilizing TOC technologies for their ability to better mimic human organs systems than 2D tissue cultures and the potential to replicate the connectivity and complexity of multiple organ systems by connecting them in series. While it is possible for individual investigators to conduct research using TOC or in-silico set-ups, the infrastructure for supporting them and assessing them is limited at NSRL, limiting research that leverages these developing technologies. It is recommended that infrastructure to support the housing and testing of TOC and in-silico technologies be expanded to facilitate future translational research.
4) **Hematology Analyzer**: A hematology device capable of measuring over 20 analytes from whole blood, including complete blood counts, in multiple species will provide a clinically relevant measure of hematological changes. The volume of blood necessary is dependent on number of parameters measured but should be optimized for relatively small volumes due to most studies being conducted in rodents.
5) **Flow Cytometry.** At NSRL a single laser flow cytometry device is currently available for use. A flow cytometer with at least four lasers is recommended to perform complex flow cytometry experiments including immunotyping.
6) **Fluorescence Microscopy System:** Microscopy techniques are limited at NSRL and do not support the advanced capabilities of time-lapse capturing and scanning of the large fields. Recently, a Keyence BZ-X800E All-In-One Fluorescence Microscope system, referred to as

KERMIT, was installed for use on the International Space Station. It is recommended that the current equipment is upgraded to be equivalent of ISS or better.

7) **Environmental Chambers (Rodent/Plant):**
   a) Rodent Environmental Chambers: The addition of rodent environmental chambers capable of replicating environmental conditions of spaceflight would be beneficial to enable better comparison and validation of the ground-based experiments.
   b) Plant Growth Chambers: Limited research on the effects of space radiation on plants has been performed. The need to grow plants in space increases as we travel deeper into space due to limitations on mass/volume/power of space vehicles. Furthermore, plants provide an important behavioral countermeasure for the astronauts when off-planet, so it is important that plant growth is optimized. To facilitate future plant studies, it is recommended that plant growth chambers are installed at NSRL.

In conclusion, the above simple yet attainable recommended upgrades (Table 2) would capitalize on the unique capabilities of NSRL and leverage state-of-the-art technologies resulting in an acceleration of research in the effects on not only human physiology but also plant biology and would address multiple space exploration risks.

	Recommendation	**Est. Cost**	**Primary Risk Usage**
Infrastructure	Neutron	$$$	Space Radiation (Carcinogenesis), CVD, Behavioral Medicine, Sensorimotor, Immune, Bone & Plants
	Centrifuges/clinostat	$-$$$	CVD, Sensorimotor, Immune, Bone & Plants
Infrastructure /Equipment	Vivarium	$-$$$	CVD, Behavioral Medicine Isolation/Confinement & Sleep
Equipment	In-vivo Imaging	$$-$$$	Space Radiation (Carcinogenesis), CVD & Bone
	Behavioral/Cognition set-ups	$-$$	Behavioral Medicine
	Tissue-on-a-Chip set-ups	$-$$	Space Radiation, CVD, Behavioral Medicine, Immune & Bone
	Hematology Analyzer	$$	Space Radiation (Carcinogenesis), CVD & Immune
	Flow Cytometry	$$	Space Radiation (Carcinogenesis), CVD, Behavioral Medicine, Sensorimotor, Immune, Bone & Plants
	Fluorescence Microscopy	$$	Space Radiation (Carcinogenesis), CVD, Behavioral Medicine, Sensorimotor, Immune, Bone & Plants
	Environmental Chambers	$-$$	Space Radiation (Carcinogenesis), CVD, Behavioral Medicine, Sensorimotor, Immune, Bone & Plants

Table 2. Summary of recommendations for establishing a NSSL. Primary risks impacted have been included but research may extend beyond these areas. A cost estimate has been included it ($<250k, 250k<$$<500k, $$$>500k) for each recommendation.

Trade names and trademarks are used in this report for identification only. Their usage does not constitute an official endorsement, either expressed or implied, by the National Aeronautics and Space Administration

Reference:

[1] L. C. Simonsen, T. C. Slaba, P. Guida, and A. Rusek, “NASA’s first ground-based Galactic Cosmic Ray Simulator: Enabling a new era in space radiobiology research,” *PLOS Biol.*, vol. 18, no. 5, p. e3000669, May 2020, doi: 10.1371/journal.pbio.3000669.