LIFE BEYOND LOW EARTH ORBIT

Report of a

SCIENCE WORKING GROUP

To

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

HUMAN EXPLORATION AND OPERATIONS MISSION DIRECTORATE

SPACE LIFE AND PHYSICAL SCIENCES DIVISION

TABLE OF CONTENTS

Image Credit: NASA

LIFE BEYOND LOW EARTH ORBIT SCIENCE WORKING GROUP

PAUL W. TODD, Techshot Incorporated, Retired, *Co-Chair*
ROBERT J. FERL, University of Florida, *Co- Chair*
*SHARMILA BHATTACHARYA, NASA Ames Research Center,
LEROY E. HOOD, Institute for Systems Biology
AARON L. MILLS, University of Virginia
GREGORY R. NELSON, Loma Linda University School of Medicine
CHERYL A. NICKERSON, Arizona State University
*C. MARK OTT, NASA Johnson Space Center
JAMES A. PAWELCZYK, Pennsylvania State University
RICHARD C. QUINN, NASA Ames Research Center
MICHAEL J. SANDER, NASA Jet Propulsion Laboratory (retired) (from January 2012)
*KASTHURI VENKATESWARAN, NASA Jet Propulsion Laboratory
CURT WITTENBERG, The Scripps Research Institute
LAURENCE R. YOUNG, Massachusetts Institute of Technology
*YE ZHANG, NASA Kennedy Space Center
*Appointed NASA field center representatives

NASA STAFF

Organizers
KEVIN Y. SATO, NASA Ames Research Center
HEATHER D. SMITH, University of New Mexico, NASA Ames Research Center
KENNETH A. SOUZA, Space Biology Project, NASA Ames Research Center
KENNY K. VASSIGH, NASA Ames Research Center
Facilitator
MARIANNE B. SOWA, Space Biosciences Research Branch, NASA Ames Research Center
Support Team
MARISSA E. ANAYA, KBR Wyle, NASA Ames Research Center
DENNIS B. LEVESON GOWER, KBR Wyle, NASA Ames Research Center
YASAMAN SHIRAZI-FARD, Space Biosciences Research Branch, NASA Ames Research Center
Workshop Attenders
JOSHUA S. ALWOOD, Space Biosciences Research Branch, NASA Ames Research Center
SALLY CAHILL, Space Biosciences Division, NASA Ames Research Center
JENNIFER FOGERTY, Human Research Program, NASA Johnson Space Center
RUTH K. GLOBUS, Space Biosciences Research Branch, NASA Ames Research Center
ANTHONY HICKEY, Arctic Slope Technical Services, NASA Headquarters
NICOLE A. RAYL, SLPSRA Space Biology Program, NASA Headquarters
JEFFERY D. SMITH, Science and Technology Projects Division, NASA Kennedy Space Center
SIDNEY C. SUN, Space Biosciences Division, NASA Ames Research Center
ELIZABETH M. TAYLOR, Space Biology Program, NASA Ames Research Center
DAVID L. TOMKO, SLPSRA Space Biology Program, NASA Headquarters

LIFE SCIENCE BEYOND LOW EARTH ORBIT

The Assigned Task

The opening charge to the Science Working Group provided by NASA Space Life and Physical Sciences Division and Ames Research Center was: "The function of this Science Working Group (termed LBLEO-SWG) is to provide the NASA LBLEO Project with scientific subject matter expertise to guide, review, and comment on:

•LBLEO science priorities and objectives
•LBLEO science requirements
•Identification of high impact basic research areas
•Addressing human exploration risks and knowledge gaps
•Integration with other space research fields
•General LBLEO scientific strategic planning
•LBLEO Roadmap
•LBLEO strategic and tactical plans"

This charge was not used as an outline but as a guide. The SWG was formed in 2016 and conducted a meeting in mid-July to openly discuss the items of the charge to the group. Additional discussions occurred via teleconference. The SWG chose to address these items within each of nine life science research areas. These areas, considered as disciplines, were self organizing and logical within the SWG based solely on science content and were not intended to align with particular NASA programs.

It was assumed that, administratively, LBLEO (Life Science Beyond Low Earth Orbit) will be a sponsored Project of the Space Life and Physical Sciences (SLPS) Program within the Human Exploration and Operations Mission Directorate. There was no attempt to make this a consensus document, rather it was considered important that the document give voice to offered inputs – both fact and opinion. Therefore readers of the body of the report should not expect consensus recommendations. As expected in such a diverse group, some SWG participants considered some disciplines more important than others. In the course of discussion and within this document, items that are already on the present program in space biology were considered for relevance, and items that are definitely not on the present program were considered. This document is therefore intended to capture broad scientific discussion that can be used as a background for roadmapping NASA Space Life Science research beyond low earth orbit.

Preamble

The space environment affords an opportunity to gain a better understanding of two important influences on biology– gravity and radiation – as well the role of extended duration missions on human interactions. To date, except for a few experiments on Apollo missions, virtually all of the space biology experiments have been conducted in or below low earth orbit. Soon new opportunities beyond LEO will be available as NASA once again extends human missions

toward cis-lunar and other deeper space locations. These opportunities will be of two kinds – first, the use of space to understand fundamental biological processes (research IN and OF space) – and second, the chance to develop and test new ways of protecting astronauts against the well-known threats of long duration space exploration (research FOR space). These two kinds of opportunities have been richly explored in low earth orbit, yet deep space presents a special extension of these opportunities. NASA's human exploration journey back to the moon and to Mars will require the development of science, technology and know-how to send humans beyond LEO and return them safely to Earth for periods of 8 months, up to 3 years, and travel at least 36 million miles from home. This science, technology and know-how relevant to beyond LEO has not advanced since the Apollo era.

Space biology and medicine will continue to advance with analysis and exploration beyond LEO. There are two significant aspects of such opportunities that are essential for basic and applied life science research. The first is the exposure to the full range of space radiation, both galactic and solar, unabated by the protection of the Earth's magnetic field. Traditionally space radiation has been studied only using ground accelerators. The second is the exploration of the "gravity continuum", from microgravity to one-G, and beyond to hypergravity. In addition to the use of variable artificial gravity centrifuges, LBLEO offers the opportunity to establish long duration reduced gravity laboratories on the Moon or on Mars. In addition, there is a synergy between these two major aspects in that the interaction between extended weightlessness and space radiation together with their effects on biology and medicine, can only be studied beyond LEO.

NASA's exploration move beyond LEO coincides in time with technical advances that will greatly enhance data return from exploration missions. Historically, most of the advances in the understanding of space biology and medicine have been derived from samples returning from LEO for processing in terrestrial laboratories. This works well in LEO, where flights to and from orbit sustain a rather constant flow of experiment up and sample down. Long term missions beyond LEO will not support regular sample return. However, substantial advances in technology, particularly in the emerging field of "'omics" enables the conduct of animal, human and plant research to occur during spaceflights with only data being returned to Earth.

At first we needed to know if humans could even survive in weightlessness beyond a few seconds. The reports of the National Academy of Sciences in the 1950s raised questions about basic physiological functions – cardiovascular, respiratory, gastrointestinal, and others. Following the successes of Cosmonaut Gagarin and Astronauts Shepard and Glenn the emphasis on survivability in space expanded to Extra Vehicular Activity (EVA), longer duration, space radiation and psychosocial issues. The Apollo successes, followed by the initial findings, from Skylab, of deconditioning associated with periods up to several months in space, lead to basic studies of space biology and medicine. The Soviet Biocosmos missions and the numerous studies carried out on plants animals and humans in the Spacelab, on the Space Shuttle, all significantly clarified the effects of weightlessness on physiological systems, the underlying nature of space deconditioning and needs for countermeasures to overcome deconditioning in weightlessness. The missing variable was mission duration – and that has been encompassed by the investigations carried out on the International Space Station – now extending up to a year. Aside from the technical and psychosocial issues associated with a long duration mission carrying astronauts as far as Mars and lasting for several years, there remains only one known critical

environmental variable to be explored – space radiation. All of the other space variables can be studied with the ISS or other satellites in LEO. LEO affords the protection of the Earth's magnetic field, which deflects most of the incoming charged particles constituting both the cosmic background radiation and the periodic bursts of solar event flux. Therefore one of the prime motivations for studying Life Beyond Low Earth Orbit (LBLEO) rests on the biological and physiological effect of radiation, combined with long duration weightlessness or exposure to gravity less than that on Earth.

The life science investigations required by LBLEO remain divided into two categories, as in the past. The first category consists of the issues of human survivability and life support. Long duration missions, with or without added radiation challenges, will require the presence of reliable generation and recycling of oxygen and carbon dioxide, generation and purification of water, and monitoring and control of the microbiological status of a closed inhabited volume. Life support systems will need extensive engineering developments regarding growth, preparation and storage of food, stability of drugs and handling of all kinds of waste. These issues, along with the problems of communication delays and crew interactions, can all be studied in LEO. However, when radiation is thrown into the mix the studies become more complex, and require missions that have orbits extending beyond LEO, including cis-lunar missions as well as those to Mars or its moons. The second category of scientific investigations which will require missions beyond LEO consists of basic biological studies of the interactions between gravity and radiation effects. Here it is desirable to be able to vary the gravitational level over the entire hypogravity range, from the free-fall of weightlessness to the pull of Earth's gravity. Cells and tissues, organs and whole animals, can be studied during long duration orbital flights of the cis-lunar variety, as well as in simple earth orbit. Provision of a centrifuge on such missions will permit the systematic imposition of g levels both lower and higher than Earth's gravity.

For these reasons, from preparation for eventual human exploration of Mars to the basic understanding of the interaction between gravitational pull and radiation on biological material, LBLEO will require multiple space missions, extended in both duration and mission profile.

FRAMEWORK

The roles of Space Biology in LBLEO Strategy were identified in a framework provided by NASA Headquarters. These five roles were identified and articulated by NASA to the SWG; however the clauses following the dashes were added by the SWG.

- Provide a framework to build a foundation of how biology adapts and changes in response to spaceflight – broad general principles founded in physical and molecular sciences
- Provide a research map that will intrigue and engage the best scientists to contribute their skills – bring in state of the art technologies, 'omics, gene editing, microfluidics
- Ensure that biological research is by design synergistic with defined needs of human biomedical program – create closer cross-discipline ties within the agency

- Build an understanding of the effect of gravity as a continuum (GAAC) on biology by sponsoring research that manipulates gravity as the independent variable – finally identify the need for artificial gravity
- Build the biological infrastructure for future exploration, openly available contributions to Space Life Sciences -- for, in and of space

The SWG was not governed by administrative expediency and it took these roles seriously in its functioning as an independent body.

DISCIPLINES

Space life science research tends to fall into three categories. Life science research FOR space: What do we need to take when we pack our bags? Life science research IN space: What significant experiments will exploit the unique advantages of the deep-space mission environment? Life science research OF space: How should we conduct the search for life and its precursors when we get there?

Nine disciplines were recognized by the Science Working Group, and all categories of research were considered in each discipline. Human risk and life-support related disciplines are recognized as research FOR space, but much of this research is translatable to Earth problems, qualifying as research IN space. Seeking and/or understanding life in the universe is the research OF space, primarily covered in the Astrobiology discipline. Summary recommendations from each discipline are listed in this section, and each of the discipline "Goals" is represented with background and specific science objectives as a brief chapter in the subsequent pages. These "Goals" are a combination of consensus and individual opinion. There are narrative synopses of the listed "Specific Science" items in each of the corresponding Goals statements.

1. Plants. It is time to get aggressive about growing food off the planet. This goal represents an excellent opportunity to exploit the extensively characterized plant genomes to reprogram major features that humans can manipulate, like growth rate, moisture requirement, disease resistance, flavor, gravity responses, photo responses, secondary metabolite levels, etc. This research occurs first on the ground and consists of unrestrained genetic engineering experiments – a great NASA opportunity -- in contained laboratories, unbridled by the fears of release to the environment and citizen objections. It would be ideal to grow food without waste – eat the whole plant, like a 10-day aeroponic beet. Sequester these products strictly for space travel only to be released to the public when identified as nutritious efficient food without risk – a 3rd green revolution. This approach creates great opportunities for synthetic biology and pulls that field forward. This approach also means giving less attention to the details of gene regulation in *Arabidopsis thaliana* and turning attention to more practical matters with equivalent rigor. Numerous plant species have been grown on orbit, some with astounding success; however, root matrix selection and design require continued exploration, and the relative merits of porous media, hydroponic seal and aeroponic mist (which is of rising interest) are still under discussion. While spectrally ideal combinations of LEDs have been identified, it would still be valuable to determine a means of using the ambient continuous daylight of interplanetary space to potentially save energy and spacecraft complexity. NASA is implementing a Passive Orbital

Nutrient Delivery System (PONDS) prototype into a flight-qualified Enhanced Passive Water Delivery System (EPWDS) for the eventual purpose of most effectively delivering aqueous nutrient solutions to the roots of plants intended for food.

2. Microbes. Spacecraft interiors constitute a built environment. Experiments over the past 50 years have shown that microorganisms respond to short duration culture in the microgravity environment of spaceflight in unexpected ways, including alterations in virulence, gene expression, resistance to antibiotics, materials degradation, and biofilm formation. However, no information is currently available regarding the response of microbes (pathogens and commensals) to long duration culture in the chronic stress of microgravity, nor in response to fractional gravity levels that will be encountered during exploration missions. Likewise, how microbial diversity changes (in the built environment and the human microbiome) during LBLEO will also be important to consider. Cyanobacteria or unicellular algae have been proposed for recycling oxygen from CO_2 and providing food at the end of their cycle; however, palatability issues will need to be solved by further research for the feasibility of crew consumption. Preparation for space travel beyond LEO is a very good reason to aggressively pursue studies to understand the impact of long duration culture and fractional gravity on interactions between the microbe, the host and the environment. This includes studies of pathogenic and commensal microbial responses (genotypic, molecular genetic, metabolomic and phenotypic), host-microbe interactions (human, animal and plant hosts), environmental microbiology (diversity and impact of microbes on vehicle integrity and onboard operating systems). The function of microbial cells can be leveraged genetically to enable their beneficial functions in LBLEO or prevent harmful functions using next generation tools of genomics and recombinant genetics. Microbiology for LBLEO could be a highly translatable exercise in the direction of practical and constructive applications of synthetic and systems biology with NASA leading the way.

3. Immune Systems. Spaceflight in LEO has been shown to measurably affect innate and acquired immune responses in humans and experimental animals, which suggests an increased risk of disease events during spaceflight due to potential immune dysfunction. While these collective data indicate an elevated disease risk for astronauts (including infectious disease, cancer and autoimmune disorders), the clinical relevance of the immunological changes induced by spaceflight remains to be established. The influence of space travel on the interactions of the immune system with the neuroendocrine axis and bone formation will need to be better understood on the basis of fundamental research. As proper functioning of an astronaut's immune system is essential in order to maintain crew health and performance throughout a long-duration spaceflight mission, immunological research will be critical for LBLEO missions to better understand if longer flight times or exposure to space outside of the protection of LEO will exacerbate the immune dysfunction that has already been well documented in International Space Station (ISS) and Space Shuttle crews.

4. Muscle and Skeletal Systems. The most conspicuous effect of prolonged weightlessness is the loss of muscle and therefore bone. This discipline is of immediate and long-standing need of intermediate (0 – 1 g) and intermittent inertial acceleration sources. It has been said that suitable facilities have been requested for over 40 years. There is widespread opinion that artificial gravity, properly engineered into the deep-space transfer vehicle, would remove most of the

uncertainty about crew fitness (strength) for a planetary landing and for the return landing, if planned, on Earth. Tools exist for the precise quantification of muscle and bone in laboratory animals and in humans. Desired is an agreement on what experiments must be done to choose the optimum acceleration levels and frequencies for an artificial gravity setting. The principal barrier is the missing facility capable of providing intermediate acceleration levels "across the gravity continuum" for studies that could be considered meaningful on orbit and on earth.

5. Cardiovascular systems. There is significant dialogue concerning the role of model organisms and the availability of human data. Only in model organisms such as *Drosophila melanogaster* (in which, despite being an invertebrate, very fundamental molecular data have been obtained) or laboratory rodents can such questions as signal transduction modifications and regulation of gene expression be obtained. Stored biological samples can and should be analyzed for changes using the best available methods of the day (e.g., and depending upon sample type, cell-free DNA, exosomes, etc.) and all future human-crewed flights include aggressive sample collection and preservation to allow for future measurement techniques to be applied downstream. In addition, serious consideration should be given to continuous (where possible) cardiovascular data collection via unencumbering wearables and other state-of-the art miniaturized instruments, especially including ultrasound. Early beyond-LEO and present-day LEO missions dedicated to cardiovascular medicine, or even medicine in general, should be considered where gathering physiologic and molecular data from crew members and research organisms is a prime driver. Mission durations and the demographics of humans who NASA and the world at large send on space missions are changing. Further, with commercial space on the rise, it is quite possible that paying customers will "beat NASA to the punch" in some cases and there the demographic will be those wealthy enough, who will likely be older. We will need genomics, epigenetics, and all elements of the rich 'omics data being produced in cardiovascular laboratories and clinics, and this field is ripe for applications of organ-on-a-chip and printed-organ research technologies. The human data that currently exist do not yet comprehensively answer the question of the effects of densely ionizing radiation at the doses present in deep space. The combined effects of microgravity and radiation will also be important to study using multicellular biological systems. Therefore, coupled with open access to low earth orbit astronaut data, there should be studies with well characterized genetic model organisms such as invertebrates and vertebrates in order to prepare for long duration deep space missions. There are solid data collection opportunities from humans and model target organisms that therefore should not be ignored.

NASA's Human Research Program (HRP) deals with human subjects, funds grants related mainly to human studies, and also deals with risk assessments for astronauts (including space radiation), sample and data collection. Cardiovascular changes for astronauts is a major topic within the HRP portfolio including risk assessments, monitoring astronauts before during and after spaceflight, sample collections and data analyses etc. To take full advantage of rapidly rising basic biological research technologies, the HRP needs to become more open to seeking answers from the Space Biology community. Indeed, these disciplines, (and not just in the cardiovascular field) need to be so tightly blended that the notion of separate "communities" ought not apply.

6. Central Nervous System. Exposures of brain tissue to densely ionizing radiation can lead to persistent deficits in cognitive functions and behaviors. However, little is known about the quantitative relationships between exposure dose and neurological risks, especially for lower doses and dose rates and among genetically diverse individuals. Acutely delivered doses of heavy-ion radiation comparable to an annual dose beyond LEO cause measurable performance deficits in rodents. Anatomical correlation of detectable histochemical and cellular events with function deficits is only beginning to emerge (e.g. dendritic spine defects), while such correlations are readily apparent in semiconductor materials. This correlation is important owing to the potential elimination of a critical function dependent on a very small number of cells (e.g. as found in the locus coeruleus). The availability of optical nanosensor technology, single-cell and in situ 'omics and "optogenetics" in viable animal test subjects should be brought to bear on this remaining urgent space radiation issue. The movement from fiction to scientific research of concepts of torpor-inducing transfer habitats for human stasis during travel to distant beyond LEO destinations in not taken very seriously. This field could benefit from the application of 'omics research to the study of estivating/hibernating animals.

7. Reproduction and development. This subject refers to animal (not plant or microorganism, covered in their respective sections) reproduction and development. The earliest of all biological experiments in orbit included embryological development, because embryos were a source of organisms that had never been exposed to earth's 9.8 m/s^2 acceleration. Today's developmental biology world offers almost infinite opportunities to explore gene expression and signal transduction pathways required for specific developmental steps and the role of inertial forces in and the effects of radiation on such steps. These include subjects as diverse as reproductive biology, wound healing, general and tissue-specific components of cell differentiation, tissue regeneration, etc. The physical-chemical environment that calls forth cells to differentiate during organ recovery, wound healing and normal tissue turnover should be of interest. Genetically well characterized model organisms can therefore play a vital role in understanding the long-term effects of deep space radiation combined with microgravity.

8. Radiation. This subject was surprisingly dominant in the discussion and writings of the SWG. NASA space radiation biology has stressed reduction in uncertainty of fatal cancer and has included neurological research projects. It is still not known if mission-critical cognitive functions will be meaningfully affected by galactic cosmic rays or whether the known combined effects of space radiation and unweighting on the immune system and bones will threaten deep-space mission success. Concern has been expressed about the modest efforts going into the cardiovascular system. The need to understand non-cancer endpoints is obvious. HRP "owns" the radiation program, as said during discussions, along with other research disciplines that could benefit from tighter integration with fundamental biological research that cross-cuts among human research, human biology and fundamental biology. To the extent that administrative barriers inhibit interdisciplinary basic research that leads to safe travel beyond LEO these barriers need to be lowered. Accurate basic space radiation studies still need to be done on the ground with a carefully vetted standard model galactic cosmic ray simulation spectrum of particles and energies. This needs to be used methodically for extrapolation from laboratory doses and dose rates to spaceflight environmental doses and dose rates in order to complement in situ spaceflight data. Antarctic balloon flights can provide more realistic exposures closer to that

which a crew will experience during transit to Mars (lower dose and dose rate, multiple particles, long duration).

9. Astrobiology. Robotic deep space missions can provide opportunities to advance both Space Biology and Astrobiology programmatic goals. To explore the common theme of *Environment and Evolution* beyond LEO, from either a Space Biology or an Astrobiology perspective, scientific tools and technologies are required and represent an area of potential coordination. A balance is needed between principles of planetary protection and the advancement of planetary life science. The high level of interest in, and importance of, planetary protection is not matched by a corresponding research budget. There has not been a detection-of-life experiment since the Viking missions in the 1970's. If a search for life needs to precede the embedding of life, then search-for-life experiments will need to be treated with more urgency. Technologies for Space Biology investigations and Astrobiology exploration have facilitated numerous unmanned spaceflight experiments with beyond-LEO potential. Continued development and application of microfluidic based technologies, planetary protection technologies, synthetic biology, and habitability research all represent critical leveraging points needed to enable the achievement of NASA's Space Biology Program and Astrobiology Program goals. "Space Biology" and "Astrobiology", which are separated by several walls in the NASA chart of organization, both should be led forward to LBLEO by similar utilization of these technologies. Facilities that provide high-fidelity simulations of planetary environments for biological research, which would seem to be a basic tool in this field, are inadequate. Additionally, there are 'omics and molecular technologies in daily use in origin-of-life research labs that could have greater visibility in the broader LBLEO environment.

TECHNOLOGIES

Several technologies and approaches that are applicable in many of the above nine disciplines arose during discussions. The desirability (and in some cases urgency) of applying these could not be overlooked. The following (in no particular order) were identified as the more conspicuous opportunities.

Intermittent Artificial Gravity. It is difficult to be emphatic enough about this. The use of artificial gravity as a countermeasure to insure crew health beyond LEO remains a subject of debate. Engineering decisions cannot wait much longer for critical data. Sustained commitment to ground based and especially flight testing of artificial gravity (AG) has been lacking. Despite numerous studies in the U. S., Russia, Japan and in Europe which demonstrate the effectiveness of AG in combating the debilitating effect of bed rest, no progress on human in-flight testing is in evidence. There is, however, hope of some progress in the use of a Japanese build rodent centrifuge expected to be available on the ISS. NASA's Human Research Program recently funded four projects that will investigate the sensorimotor, cardiovascular, visual, musculoskeletal, and behavioral responses in humans to intermittent artificial gravity during bed rest. These four projects will complement seven studies recently selected by the European Space Agency (ESA). The projects will evaluate the possible benefits of artificial gravity on human health in response to the detrimental effects of spaceflight as simulated in a bed rest analog. This work is helping NASA develop the resources and countermeasures necessary to ensure

astronauts remain healthy as we venture beyond low-Earth orbit and head out to study an asteroid and eventually Mars. The studies will be conducted in the *:envihab* facility located in Cologne, Germany, a state-of-the-art facility for conducting ground-based research in support of spaceflight. Projects will make use of its short-arm centrifuge, and subjects will undergo 60 days of 6° head-down tilt bed rest with intermittent centrifugation. The ambulatory periods two weeks prior to and after bed rest will allow for baseline data collections and recovery after bed rest. These studies should form a springboard for pre-LBLEO on-orbit evaluations using long-term rodent centrifugation and human short-arm rotation studies that cross-cut the disciplines – cardiovascular, musculo-skeletal, immune and CNS. They will, in any case, require validation and demonstration with an in-flight human centrifuge and exercise device capable of testing the effectiveness and acceptability of AT at various g-levels and duration.

Synthetic Biology. NASA has developed considerable interest in synthetic biology since 2010, with a mission "to provide robust biological tools and technologies to sustain human activities across the solar system for the benefit of exploration, science and the economy." J. Craig Venter has expressed interest in this aspect of LBLEO. Areas of interest include in situ resource utilization; biomembrane-based filters; bio-based production of materials for advanced manufacturing; biological 3-D printing; bio-mining to obtain minerals from planetary surfaces or spent electronics; production and purification of "on-demand" pharmaceuticals; food production; life support; and tools to address astrobiology questions. Specific applications to LBLEO are numerous and include recycling human waste into nutraceuticals and materials (closing the loop for long-term space travel), a flexible synthetic biology tool kit that can be used to supply a large variety of materials for the mission, designed microbes for digestion of solid waste, plants for growth chambers for food production, atmospheric trace contaminant removal, design of a cellular system suitable for resource utilization on extraterrestrial planetary surfaces, chemical materials manufactured from 3d-printed synthetic biology arrays, genetically engineering microorganisms to produce bionutrients to supplement crew food supplies, and genetically engineered organisms for biosensing or bio-manufacturing. The notion of "built organisms for a built environment" means constructing organisms to function for the benefit of humans beyond LEO or constructing model organisms to optimally evaluate a particular stress by manipulating their resistance and/or sensitivity to the stress. This is a well-established approach in various microorganisms (bacteria and yeast species) as well as *Drosophila, C. elegans*, and mice. These approaches provide obvious advantage in amplifying the effect and/or evaluating mechanisms of resistance to various stresses including radiation and gravitational field variation. These approaches can be combined with retrospective 'omics and systems-biology analysis to evaluate the impact of genetic manipulation. Of course, these approaches currently require sample recovery and, therefore, a return mission. That is likely to change in the not-too distant future. Much of this type of analysis requires human intervention to collect samples and preserve them, especially when mammals are involved. Microorganisms and some types of metazoans, are already amenable to robotic sample collection and preservation. These approaches could be applied to studies of biofilm evolution; similar studies are being performed in land-based studies of biofilm development and evolution – potentially useful in water recycling. The widespread availability of gene editing technology (absolutely any gene), especially the CRISPR-Cas9 technology, should revolutionize the way we prepare a living environment for LBLEO, including extraterrestrial settlements. This subject seems to have waned in SMD's astrobiology programs. Breakthroughs facilitating living in space are possible.

'Omics. Taking broad applied 'omics approaches to dissecting the impact of deep space travel on organisms ranging from microbes to humans is strongly urged. Due to the constraints of the scheduled missions, there will be limited opportunity for return of biological material due both to the sparse timeline and the constraints on payload. Those facts and the power of integrated 'omics approaches (genomics, transcriptomics, metabolomics, etc.), leads to the belief that application of those approaches to material collected during deep space missions would be the most likely to yield information at a density sufficient to justify the cost of their application. It is very clear that the power of such analyses and their sufficiency to evade the necessity for preformed hypotheses make them particularly appealing for retrospective analysis of the impact of spaceflight on the biology of humans and other organisms. These approaches can be applied to collect data from experiments that are designed in advance using microorganisms, invertebrates including *C. elegans, Drosophila*, plants and even small mammals but they can also be applied retroactively to those organisms as well as to human subjects who travel on those missions. Furthermore, the richness of the data renders the data invaluable for reanalysis to address hypotheses proposed post flight and warrants sample collection and preservation done with consideration of future needs. Because data regarding human activity are being recorded throughout the mission, samples collected throughout the mission may be invaluable in evaluating the physiological and genetic impact of events, planned or otherwise, that occur during deep space travel. If conditions allow, these analyses could be performed in real time. The application of 'omics across all of the Goals is also worthy of consideration since the same datasets collected during spaceflight to address one Goal will likely address questions across Goals.

Two approaches to the application of these 'omics technologies are envisioned. First, broad ranging analysis of the genome, transcriptome, metabolome, proteome and microbiome can provide valuable insights into the state of a system and to changes that occur during deep space flight. The data provided by analysis of those samples, when evaluated in the context of the vast data sets being collected by many labs world-wide will provide an array of conclusions and new hypotheses. Some of those hypotheses might be addressed by reanalysis of the same data set whereas others may require that new sample and data collection approaches be employed on future missions. Second, experiments may be conceived in advance driven by hypotheses proposed pre-flight. These enable manipulation of the system (manipulation of environmental conditions including g-force, radiation, all nature of physiological manipulation, etc). Importantly, those manipulations need not preclude retrospective analysis of the samples as a test for other hypotheses. Finally, each of these approaches can be applied to model and environmental organisms as well as astronauts. All of these potentials can be fulfilled by extensive utilization of the NASA GeneLab open integrated repository and bioinformatics system for analysis and modeling. This will enable the discovery and validation of molecular networks that are influenced by space conditions through ground-based and flight research using next generation "omics" technologies and will engage the broadest possible community of researchers, industry and the general public to foster innovation. However, to be successful in this endeavor, it is critical that 'omics based experiments be done in the context of hypothesis-driven goals to facilitate practical interpretation and integration of 'omics data into a biologically meaningful, comprehensive and mechanistic understanding of cellular/molecular responses (and not just doing -omics for the sake of -omics and then depositing the data in a database that cannot

be effectively utilized). 'Omics approaches are sufficiently versatile and effective to impact virtually all disciplines considered in this report.

Genome wide association studies (GWAS). To characterize genetic susceptibilities of all organisms to the radiation and microgravity environment beyond LEO, GWAS need to be considered. Most observable phenotypes in invertebrates and vertebrates are complex in nature and result from the quantitative interactions among multiple genes whose expression can often be affected by the environment. Well characterized genetic models that can be studied in sufficient numbers in this novel environment will be critical for an unbiased screen with quantitative genetics for the discovery of new and interacting gene loci and pathways that are relevant for LBLEO. Simple model organisms like *Drosophila* which have highly homologous systems to mammals and can be grown in large genetically identical and well characterized populations will be important for quantitative trait loci mapping for spaceflight where volume and mass are limiting. Such information will be critical for extrapolation to humans and to predict physiological responses to BLEO and for future countermeasure development and testing.

'Omics, Data and Crew Privacy. Genomic analysis (as well as other 'omics approaches) are generally anonymized effectively due to the large numbers of individuals undergoing the analysis. This is not really possible in the LBLEO case since the human sample sizes are small. One solution to this problem is to separate the genomics data and data analysis from those managing flights and selection of the crew members. SWG members would like to see an open source of crew data, suitably de-identified to preserve crew anonymity, in a GeneLab-type accessible database or in the GeneLab database itself. Such an approach should reduce barriers between medical science and basic biology, which, in the SWG view, may inhibit the elucidation of biological mechanisms that drive health risks in deep space exploration. More than 700 humans have spent time in LEO. We should start by making their health data available to researchers who understand clinical medicine, gene expression and fundamental physiology, for example. The crew could be selected based on criteria independent of those derived from this analysis, and the analysis of data, which is expected to be performed after the fact, should be considered independent of future crew selection. Trust in that point will undoubtedly be a prerequisite for approval for the analysis by potential crew members. This is, of course, easier said than done. The 'omics analysis of humans may require that this matter be resolved. There would be missed opportunities should it be avoided for these reasons. All humans who participate in space flight at public expense should be required, as a condition of participation, to have their de-identified physiological 'omics medical data available for research. The risk of medical disqualification is a big factor, but this needs to be expected. There are plenty of individuals who would gladly volunteer to be test subjects, especially as commercial access beyond LEO develops, as expected, in the future.

Micro-Miniaturization. New bio-analytical instruments suitable for launching to and operation in LEO are becoming available at an almost monthly frequency. NASA's Wetlab projects have been attempting to follow this trend. Coming with each new instrument is a reduced amount of effort required to adapt it for space flight. Indeed, a Nanopore (single-molecule) DNA sequencer has been tested on ISS. Thanks to powerful ELISA (Enzyme-Linked Immunosorbent Assay) adaptation to microfluidic systems thousands of blood proteins can be quantified without an

electrophoresis step. A hand-held microelectronic microfluid blood cell analyzer can be expected. These developments impact LBLEO in two ways: Analytical data can be collected *in space*, including beyond LEO, without any on-the-ground involvement, and the chemical reagents, not the instrument, constitute nearly all of the upmass. The selections from among these technologies will depend on LBLEO priorities.

3-D Tissue Engineering. Three-dimensional (3-D) tissue culture models of human and rodent tissues/organs, including vascularized tissue constructs capable of transplantation have been achieved by a variety of different techniques (including bioprinting, optimized suspension culture, organ on a chip, etc). Such 3-D constructs are also invaluable as predictive disease models and for understanding how human tissues respond to the unique environment of spaceflight and its associated stresses, including fractional gravity and radiation. Thus, the potential for the robustly growing 3-D tissue engineering field to crew health cannot be overlooked. Would travelers beyond LEO wish to pack this technology in their bags? It needs to be determined whether there will exist mission-critical mishaps that are countered by this technology and whether, within a decade, it would be feasible to adapt this technology for beneficial applications, including the support of surgical management of crew health problems should they arise. This technology has heavy regulatory implications and needs a plan and a policy.

In Situ Resource Utilization (ISRU). There are numerous Space Biology opportunities associated with ISRU. There has been brief consideration of bio-mining the moon, orbital planetary atmospheric resource mining, bio-mining resources for printable electronics and similar undertakings, some of which have been partially sponsored by NASA Innovative Advanced Concepts (NIAC). The role of Space Biology in ISRU, a very significant component of beyond LEO planning, has been given too little attention.

Management and Administration. The LBLEO SWG recognizes a need for integrating space life science research more intimately across the agency and within government. In view of the selection of disciplines canvassed above, the Human Research Program (HRP) and the management of crew health the following characterization of the HRP and its research program HERO is noted (quoting): "Human Exploration Research Opportunities (HERO)–2016 consists of applied research in support of NASA's Human Research Program (HRP). The HRP contains six Elements: Space Radiation, Human Health Countermeasures, Exploration Medical Capability, Behavioral Health and Performance, Space Human Factors and Habitability, and International Space Station Medical Project. Fourteen disciplines or areas support the Program: the Behavioral Health and Performance, Bone, Cardiovascular, Extravehicular Activity, Immunology, Medical Capabilities, Muscle, Nutrition, Pharmacology, Radiation, Sensorimotor, Advanced Food Technology, Advanced Environmental Health, and Space Human Factors Engineering. This covers all aspects of research to provide human health and performance countermeasures, knowledge, technologies, and tools to enable safe, reliable, and productive human space exploration." In addition to collaborating more intensely with HRP there are also opportunities in the Advanced Exploration Systems Division, such as small satellites which should lead to deep-space research potential.

SOME HIGHLIGHTS

From the deliberations, conversations and writings of the SWG, especially related to cross-cutting subjects, it appears that it would be beneficial if certain administrative practices were modified. Some that were noted are as follows:

- "Ownership" of research subject matter within specific Divisions and even specific Mission Directorates was a concern of several SWG members who would like to see enhanced cross-utilization of research results and technologies as a means of enhancing interdisciplinary research.
- The lack of access to human orbital experience (crew) data in a Genelab type paradigm , involving an epidemiologic group exceeding 700 in number (all of whom can be de-identified for scientific purposes), is a deterrent to discovering cause-and-effect relationships, genetics of responses to the space environment, and integrating fundamental principles of human space biology for applications beyond LEO.
- The reluctance to create intermediate-g-level (centrifuge) opportunities is preventing studies that may be found essential to deep space survival.
- Well designed and tested synthetic biology approaches should not conflict with the evolving principles of planetary protection.
- A reasoned approach to genetically modified organisms could lead (cautiously) to the development of "built organisms for the built environment" and the progressive translational applications thereof. The behavior in low gravity and the disease-bearing potential of "built" organisms should be understood. NASA has an opportunity to safely assume leadership in this field owing to beyond LEO requirements.
- Strong traditions averting human discrimination of any kind will need to be modified in the selection of human travelers to deep space, and the powerful tools of genome analysis need to be cautiously admitted to human research and the crew selection process.

Mission success in deep space exploration requires an extremely robust Built Environment. The Built Environment encompasses many living things besides humans. The human inhabitants of the Built Environment need to be selected with as thorough a proof of their acceptability as science can provide, including full genomic analysis. The other living things should be "built" as well. Consider, for example, aeroponically grown rapid-cycle plants providing non-monotonous full nutrition using the whole plant without waste, enteric organisms that provide resorbable daily required vitamins, uncontaminated biofilms to aid the digestion of human waste.

GOALS

Each of the nine disciplines identified is represented by a Goals report in the following nine sections. Here the term "goal" is used to define the points toward which the SWG believes each life-science discipline should be pointed for the successful execution of adventures Beyond Low Earth Orbit. Each Goal was developed by a small group of authors selected from the SWG, who organized their presentations in terms of *Background* and *Specific Science*. These were then reviewed by as many members of the SWG as were willing.

GOAL 1—DETERMINE THE PRIMARY IMPACTS OF DEEP SPACE ON PLANT BIOLOGY AND CHART THE IMPLICATIONS FOR BIOREGENERATIVE LIFE SUPPORT IN DEEP SPACE EXPLORATION MISSIONS.

This goal seeks to understand plant biology as it may exist beyond the proximity of Earth and outside the protection of the Earth's magnetic field, particularly in vehicles and habitats that are envisioned in the exploration roadmap. This goal therefore focuses on the basic and fundamental impacts of deep space on plant physiology, plant reproduction and plant genetics. This goal also highlights the engineering roles that plants serve as key part of bioregenerative life support concepts. Therefore, the concepts encompassed in this goal contain, by definition, both fundamental and applied science.

BACKGROUND

Plant biology continues to occupy an important and unique position in space biology research. As developmentally complex, eukaryotic model organisms, plants offer tremendous opportunity to advance understanding of life adaptation to extraterrestrial environments. As photosynthetic life forms capable of recycling human wastes while producing oxygen, water and food, plants offer the potential to help complete and augment the human life support loop. Thus plant biology informs the LBLEO effort on two main fronts, their participation in understanding basic biological adaptation to space and spaceflight environments, which in turn better informs the use of plants in life support. Those two intertwined aspects, understanding biology and using that understanding to support further exploration, form the basis for the key observations and questions presented in this chapter.

Current recognition of the importance of this position relative to life support is reflected in initiatives such as the HEO SLPS is investment in VEGGIE testing on ISS and HEO promoting Advanced Exploration Systems (AES) with Orbitec to design and test concepts for a "Greenwall' system for transit and habitation modules. This is in part melding plant systems and water storage systems that could also provide radiation shielding. The Space Technology Mission Directorate (STMD) has issued a request for proposals for a Space Technology Research Institute (STRI) with one topic being "Biomanufacturing", and a component of that would be food production. The German DLR led EDEN project with an analog plant growth testbed at Antarctic Neumayer Station advances the concepts of plant production in support of extreme human endeavors and ESA continues with their MELISSA project. Historically the Russians

have pursued closed human-plant systems, and the Chinese are working with their Lunar Palace and related ground testing of plant human support systems.

Advances in plant space biology over the past decade have greatly informed the physiologically adaptive processes of plants in accommodating to spaceflight, and have begun to unravel the various effects that are caused by microgravity *per se* or the broader aspects of the spaceflight environment. Nonetheless there remains a need to understand the fundamental environmental conditions for plant growth in spaceflight situations. To date, most plant experiments have involved growth in simulated Earth conditions aboard spacecraft of one kind or another. The programmatic cost of transporting infrastructure (structure, lights, atmosphere) and creating stable 1g environments in vehicles will be prohibitive. Thus understanding the basic conditions (soil/modified regolith, minimal quantity and quality of light, minimum O_2, CO_2, N_2, fundamental water movement and water use efficiency in different atmospheric pressures) for growth, reproduction and fruit development is critical. These data would be useful regardless of where we go, as regardless of the destination, we would understand what minimal infrastructure we would need depending on what conditions are there.

All of these biological considerations, especially for the use of plants in life support, need to trade favorably against other Environmental Control and Life Support (ECLSS) technologies. Short term stability and long term sustainability are prominent end goals that draw upon fundamental space biology principles while contributing to exploration capabilities.

SPECIFIC SCIENCE

Note: The following items need to be gathered into specific objectives and example investigations, provided that is the chosen format.

1. Advance knowledge and basic understanding of plant development, metabolism and their interaction with environments they encounter in space to enhance yield potential, stability and quality of food plants for long term space missions.
2. Apply synthetic biology. Engineering processes in plants to overcome marginal conditions in space (e.g. N fixation in vegetables, crops that can more efficiently utilize limited nutrients/water, sentinel plants for environmental monitoring?).
3. Identify/develop new plant cultivars suitable for spaceflight. Approach this more from the perspective of plant breeders. Can we develop crop selection strategies on Earth that lead to improved plant cultivars that can be used in space colonies? In other words, ground selection strategies for breeding space plants. Can we mine omics data sets to design selection or plant breeding strategies.
4. Translate research that will take basic discoveries at the cell/molecular level into developing plant growth systems/habitats/hardware for spaceflight.
5. Make full use of advances in genomics, genome editing, ecophysiology and modeling to design advanced life-support (ALS) biological systems.
6. Determine effects of minimal cultivation conditions on nutritional quality of plant materials for human consumption. Determine how the environmental conditions interact with each other and how we might be able to adjust/modify specific conditions and use

that information to select key genes for modification of plants for optimal quality and quantity of edible biomass, customizing for location.

7. Modify soil substrate, P, K from regolith, detoxify regolith or design plants to be resistant to pollutants, extract and use N from urine. Depending on atmosphere, employ modifications to increase O_2 to initiate plant production (i.e. Mars)
8. Modify plants and/or microbes to make high quality products for human need (antibiotics, vitamins, micronutrients, nutraceuticals, pharmaceuticals etc.)
9. Focus should be given to modifying crop species to deliver spaceflight specific nutrition (e.g., radiation and bone density loss countermeasures – fresh plums and high-antioxidant berries).
10. Breed/manipulate for pharmaceutical/nutraceutical applications. In addition to their contributions to food, oxygen, CO_2 scrubbing and water reclamation, crops can also contribute from a pharmacological perspective. This is a longer-term goal, nonetheless, the ability to 'grow' and tailor medicine and nutrients could become a real plus further out.
11. In Situ Resource Utilization and Biomaterial/textile Production: Beyond food, plants can be used for fibre production and other biomaterial production (e.g., molecular farming). Plants could be used to harvest carbon (and other elements from regolith) to make fibres that could act as reinforcing material in concrete used to 3D print habitat structures (just one of many long term application examples). Again, we can focus on plant modifications, but application development will also be key to this topic area. How we increase/better use non-edible biomass for other materials/products.
12. Continued improvement (reduction) of system mass, power, volume, and reliability
13. Better electric lighting and / or reduce wasted light
14. Use of sunlight where possible
15. Smaller (volume efficient) crops
16. Higher harvest index, less waste in the crops used
17. Improved nutritional attributes
18. Fit plants and horticultural systems into hypobaric settings (e.g., 54 kPa suggested for some Mars missions that have frequent EVAs—lower pressure reduces gas loss associated airlock events for EVAs)
19. Plant Response and Adaptation to Radiation. With much past attention directed to mutations and developmental abnormalities in irradiated seeds, the effects of cosmic radiation on living plant physiology are largely unknown, there is a real need to know how they will respond, and how to modify them to respond properly if need be, to deep space radiation environments. This can only really be done by conducting the experiments in a deep space setting or high-fidelity cosmic-ray spectrum simulation.
20. Understand microbial communities associated with plants in space systems
21. Gather data on plant / human interactions—use space habitat analogs

GOAL 2—AN INTEGRATED APPROACH TO DETERMINE THE IMPACT OF DEEP SPACE MISSIONS ON MICROBIAL RESPONSES AND DIVERSITY AND THE RELATIONSHIP TO CREW HEALTH AND VEHICLE INTEGRITY

This goal seeks to address the wide array of microbial responses and diversity as they pertain to microbial interactions with the crew, the vehicle, and other microorganisms during spaceflight missions beyond low earth orbit. This goal includes alterations in genetic, molecular genetic and phenotypic characteristics, including virulence and pathogenic properties of individual microorganisms and microbial consortia, as well as changes in and interactions of microbiomes with the crew, the vehicle, and the vehicle systems. The use of model microorganisms, real time data collection, and advanced automated hardware in these studies is considered critical to successfully accomplish these goals, as is the need for standardization and inclusion of appropriate controls in spaceflight experiments.

BACKGROUND

Innovative insight into microbial behavior has often arisen from assessing their properties in many extreme environments. Spaceflight research platforms are no exception and NASA has been performing microbiological research and operationally monitoring the crew and vehicles for microbial diversity since the Agency's inception. Early research experiments targeted basic microbial physiology to address issues that could provide insights about how missions in space might affect the health of crewmembers. However, as spacecraft and technology have advanced our capabilities, the spectrum of microbiological topics has expanded to provide insight that not only advances NASA's goals but also is of direct value to the scientific community and general public. Notably, over the past 10 years, spaceflight experiments have elucidated novel molecular mechanisms that begin to explain the unique responses of microorganisms to culture in microgravity that have been observed for over 50 years. Importantly for NASA future LBLEO spaceflight missions, these recent experiments have provided both basic and applied knowledge, such as confirming the risk of altered virulence in certain pathogenic microorganisms, such as *Salmonella enterica* serovar Typhimurium, transient increases in antibiotic resistance of some bacteria, and identification of global regulators of these responses which could be used to potentiate drug/vaccine development, and synthetic biology applications to enable future spaceflight missions. These recent studies have also demonstrated novel production and architecture of microbial biofilms unique to the spaceflight environment. Other research into the vehicle microbiome has confirmed and expanded operational microbiological monitoring showing a prevalence of human associated microorganisms, including opportunistic pathogens. Recent findings from crew microbiome studies are set to reveal spaceflight-induced changes in microorganisms associated with the crew. Collectively, these findings have advanced the field and laid the foundation for future modern microbiological research beyond low earth orbit. One interesting area that needs to be explicitly addressed is the impact of human activities on the microbiome fingerprint of the built environment. Humans may act as vectors for microbial impacts on the environment that is traceable.

Microbiological research is well suited for LBLEO. Experiments can be performed using small volumes, can fully utilize the broad array of rapidly improving biotechnology, and can be designed to operate with or without human intervention. Moreover, microorganisms replicate quickly and are thus ideally suitable for multigenerational spaceflight longitudinal/evolutionary studies. In addition, understanding microbial mechanisms and responses can be translated to other types of cells, including their mammalian successors, since more than one third of human genes have their origins in bacteria. The ability to use bacteria and other microorganisms as surrogates for mammalian cells provides the flexibility to investigate cellular responses to a myriad of environmental stressors that could not easily be performed or would be inappropriate for animal or human testing. While investigations using a range of microbial species can provide a broad understanding of the molecular and physiological alterations induced by spaceflight, the use of model organisms is critical to provide clarity into how the spaceflight environment affects the evolutionarily conserved nature of these response(s). This knowledge is especially important for new paradigms to mitigate those responses with potential negative implications for spaceflight, such as increased virulence or altered biofilm formation.

Model organisms should be readily capable of building upon previous spaceflight data and will readily improve our knowledge to the benefit of crew health or vehicle integrity benefit. Toward that goal, the best-characterized of all microorganisms in response to spaceflight and spaceflight analogue culture, especially in support of crew health, is the foodborne bacterial pathogen *Salmonella enterica* serovar Typhimurium. Notably, studies using this model pathogen have shown that culture in spaceflight and spaceflight analogue conditions globally alters its virulence, pathogenesis-related stress responses, transcriptomic and proteomic expression profiles. As a leading cause of gastrointestinal and systemic disease worldwide, *S.* Typhimurium imposes an enormous health and socioeconomic burden. *Salmonella* is also the best-characterized pathogen in terms of its physiology, growth, genetics, and molecular biology, and is thus an ideal model microbe for these studies. Moreover, there are many bacterial pathogens that gain access to animal hosts in a manner similar to *Salmonella*, and since the genetic attributes to achieve this route of infection have been conserved during microbial evolution, it is thus not surprising that the use of *Salmonella* in spaceflight experiments has already shown broad applicability in detailing how other microbial pathogens respond to the spaceflight (and spaceflight analogue) environment. From NASA's perspective, *Salmonella* is considered a potential source of infection during spaceflight that could incapacitate crewmembers during a mission. Due to its route of access through spaceflight food, NASA specifically tests for *S.* Typhimurium prior to flight and has previously disqualified food destined for the ISS based on the isolation of this microbial pathogen. In addition, *S.* Typhimurium has been isolated from the STS-108 crew refuse.

In addition to the study of microorganisms that are potentially harmful to the crew or the vehicle and its systems, many aspects of microbiological research address ways that microorganisms could benefit spaceflight missions, such as the development of probiotics or bioreactors that convert waste into usable products. This approach also includes genetic engineering of microbes for beneficial purposes for spaceflight applications, as well as understanding the impact of spaceflight on microbial population diversity in the spacecraft and crew microbiome. This broad spectrum of benefits leads to likely translation of basic research to applied purposes, including operational activities.

The issues discussed in this section have substantial overlap with Space Biology and Astrobiology, discussed in Goal 9, calling attention to what concerns our understanding of the importance of microbiome and its many implications across disciplines.

SPECIFIC SCIENCE

1. Determine if spaceflight induces changes in diversity, concentration, and/or characteristics of medically significant microorganisms associated with the crew and environment that could affect crew health (HRP Knowledge Gap MICRO-02)
2. Determine which medically significant microorganisms display changes in the dose-response profiles in response to the spaceflight environment that could affect crew health (HRP Knowledge Gap MICRO-03).
3. Determine how physical stimuli specific to the spaceflight environment, such as fractional gravity, induce unique changes in the dose-response profiles of expected medically significant microorganisms (HRP Knowledge Gap MICRO-04). This understanding includes interconnections between physical and biological stressors associated with LBLEO spaceflight-induced alterations in microbial responses and host-microbe interactions that could negatively impact or benefit crew health. Included are studies aimed at:
    a. Determination of molecular, cellular and biomechanical/physical regulators of LBLEO spaceflight-induced regulation of microbial responses and host-pathogen interactions.
    b. Determinations as to whether LBLEO spaceflight-induced changes are a direct (gravity-sensing) or indirect (ex. fluid shear, mass transfer, hydrostatic pressure) effect.
    c. Investigations into the extent of conserved, common cellular spaceflight response mechanisms in microbial and human cells. Are cells hard-wired to respond to the beyond-LEO conditions?
4. Determine the efficacy of current countermeasures and the need for countermeasure development based on changes in microbial populations and characteristics (HRP Knowledge Gap MICRO-01). These countermeasures would include vaccines, antibiotics, probiotics and other therapeutics, as well as disinfectants.
5. Determine the impact of partial/fractional gravity like those encountered in environments of the moon, Mars and other planets, and deep space to supplement microgravity studies in order to understand the degree to which microorganisms are impacted by gravity (or environmental conditions created by a lack of gravity).
6. Determine the impact of microbial infection (including viral reactivation) during LBLEO missions. All evidence of spaceflight-induced changes in microbial virulence and pathogenesis-related characteristics is from LEO models.
7. Understand how the existing evidence of LEO spaceflight and spaceflight-analogue induced changes in virulence and pathogenesis-related characteristics would translate into microbial risk assessment and clinical relevance for missions beyond LEO.
8. Understand how LBLEO mission design (including mission duration, food source, and life support systems, human-vehicle interface, etc) would impact microorganisms and their interaction with the host.

9. Characterize virulence changes in microbial pathogens, alone or in the context of mixed microbial co-cultures, to understand the impact of LBLEO spaceflight on crew health risk. For example, do the characteristics of a single microbial species in a mixed consortium (like the gut microbiome) adversely change in ways that the same species by itself, would not?
10. Understand the clinical implications for astronauts during missions beyond LEO, through mechanistic investigations into host-microbe interactions using microorganisms (including relevant mutant strains) and hosts with a dysfunctional immune response (including vertebrate and invertebrate animals, mammalian cells, or plants).
11. Determine alterations in the human-associated microbiome in the crew before, during and after spaceflight and their impact on crew health.
12. Understand the clinical relevance of potential LBLEO spaceflight-induced alterations in immune function on infectious disease outcome – *i.e.,* cause and effect.
13. Understand the impact of LBLEO spaceflight on the host tissue microenvironment that could change host-microbe interactions (*e.g.,* intestinal absorption) and thus alter host immunity and infection potential.
14. Understand the effect of sex/gender on infectious disease risks in flight. This is important as males and females differ in the intensity, prevalence and pathogenesis of microbial infections.
15. Characterize the effects of short and long-term duration LBLEO spaceflight on genotypic, molecular genetic, and phenotypic responses of microbial pathogens and commensal microbiota. These studies are needed to understand both transient and heritable changes in microbes and host-microbial interactions in LBLEO. This includes use of *omics*-based approaches (genomics/epigenetics, transcriptomics, proteomics and metabolomics). All -*omics* studies generating large data sets should be done in the context of hypothesis-driven goals and standardized conditions to facilitate practical interpretation and integration of this data into a comprehensive and mechanistic understanding of cellular and molecular responses.
16. Use eukaryotic microbes such as the yeast, *Saccharomyces cerevisiae,* to characterize and calibrate the effects of the environment as a biosensor. “Humanized yeast” cells can also be utilized in order to test the response of human genes to such environments. Use of fully automated payloads in long duration BLEO missions will facilitate studies with this robust microorganism.
17. Determine the impact of LBLEO spaceflight on quorum sensing and microbial biofilm formation.
18. Perform longitudinal studies into microbial diversity of the spacecraft during missions beyond LEO.
19. Understand how LBLEO mission design would impact microbial interactions with spacecraft materials and onboard operational systems.
20. Understand how the spaceflight environment, including choice of cultivation method (hydroponic, aeroponic) impacts pathogenicity of microbial plant pathogens.

21. Explore the possibility of carrying dried packets of engineered microbes and their media as a lightweight potential source of a variety of antibiotics, medicines and/or emergency foods when cultivated in simple fluid media bags.

GOAL 3— AN INTEGRATED APPROACH TO UNDERSTAND THE IMPACT OF DEEP SPACE MISSIONS ON IMMUNE SYSTEM FUNCTION AND THE RELATIONSHIP TO CLINICAL DISEASE

This goal seeks to understand alterations in immune system function that could lead to weakening, dysfunction and compromised defenses during spaceflight missions beyond low earth orbit. Immunosuppressive effects and immunological dysfunction have been well documented in astronauts during short and long duration low earth orbit (LEO) spaceflight and are a major concern for the health of future deep space travelers. Recent studies of crew members from long-duration LEO space missions have indicated the potential for immune system dysfunction in two key areas, 1) immune hyperactivity (associated with increased risks for hypersensitivities or autoimmune disorders), and 2) immune hypoactivity (associated with increased risks for infectious diseases, viral reactivation, cancer, and other disorders). During deep space missions (operationally referred to as life beyond low earth orbit/LBLEO), the crew will be exposed to a unique combination of stressors, including fractional gravity, radiation, prolonged isolation and confinement in environmentally closed systems, altered nutrition, altered microbial flora, oxidative stress generators, and disrupted circadian rhythms, all of which can negatively impact immune system function at the cellular, mucosal and humoral levels. Collectively, these factors can provoke an imbalance between immune system homeostasis and dysfunction, with implications for increased risk of infectious disease, autoimmune disease, cancer and other conditions due to weakened defenses. It is thus imperative to understand how deep space impacts the immune system at the innate, mucosal and adaptive levels, the mechanisms behind these changes, and the relationship to clinical disease in order to define appropriate countermeasures to mitigate immune related health problems. This goal accordingly integrates the basic and fundamental impacts of deep space/LBLEO on the immune system, and has strong associations with and implications for a myriad of scientific disciplines, including infectious disease, microbiome, nutrition, radiation, cancer, and physiological wellness. The use of model organisms (including vertebrate and invertebrate animals), defined human and animal cell types (including those used for 3-D cell cultures), real time data collection, and advanced automated hardware in these studies is considered critical to successfully accomplish these goals, as is the need for standardization and inclusion of appropriate controls in spaceflight experiments. With regard to directly analyzing human immune responses—there are now sensitive assays requiring just a drop of blood (from a finger prick) that will allow one to assess all the major chemokines and lymphokines. For example, the company O-Link (Uppsala, Sweden) has a glass slide with 92 ELISA assays for the chemokines and lymphokines (requiring just 2 µl of sample) and it can be read by a simple scanner. We have used these assays in our scientific wellness project (now spanning almost 2000 individuals) and found that they are incredibly reproducible and precise. If blood samples are obtained periodically from the astronauts, these analyses will give an accurate, direct and dynamical picture of how each astronaut's immune system is responding to the collective environmental challenges of space, all in the context of their individual genetic make ups.

BACKGROUND

Spaceflight in LEO has been shown to measurably affect innate and acquired immune responses in humans and experimental animals, which suggests an increased risk of disease events during spaceflight due to potential immune dysfunction. While this collective data indicates an elevated disease risk for astronauts, the clinical relevance of the immunological changes induced by spaceflight remains to be established. As proper functioning of an astronaut's immune system is essential in order to maintain crew health and performance throughout a long-duration spaceflight mission, immunological research will be critical for LBLEO missions to better understand if longer flight times or exposure to space outside of the protection of LEO will exacerbate the immune dysfunction that has already been well documented in International Space Station (ISS) and Space Shuttle crew.

Spaceflight effects. LEO spaceflight-induced changes to the immune system have included alterations in lymphoid tissue, the number, proliferation and function of immune cell populations involved in innate immunity (neutrophils, monocytes, macrophages and NK cells), adaptive immunity (B and T lymphocytes), and the production of cytokines and immunoglobulins. Furthermore, spaceflight-induced alterations in astronaut blood samples have shown that neutrophil phagocytic and oxidative functions are diminished, as is the ability of monocytes to phagocytose bacteria and subsequently elicit an oxidative burst and degranulate. Natural killer cell cytotoxicity has also been shown to be diminished by spaceflight, as has the production of interferon (IFN). In addition, there is also evidence of a persistent low level of inflammation in astronauts during long duration LEO spaceflight, with elevated levels of inflammatory cytokines present, suggesting that multiple physiological adaptations persist during extended LEO, including inflammation and leukocyte recruitment. Reactivation of latent herpes viruses, including Varicella Zoster, Epstein Barr and cytomegalovirus have also been repeatedly reported in LEO, an outcome associated with decreased immune function. While initially considered a function of launch and landing, multiple lines of evidence indicate that this dysfunction persists throughout 6 month LEO missions aboard ISS. As noted above, there is the possibility of following and thus assessing both adaptive and innate individual immune responses (92 cytokine and chemokine levels) over time from a finger prick of blood. Thus simple and direct experiments can follow the changes in individual astronaut immunity. The potential that this chronic alteration in the astronaut's immune system could persist during LBLEO missions strongly indicates that more research is required to better understand how to protect crew health and ensure mission success, as well as to use these findings to benefit the general public.

Of further relevance, a recent gene expression profiling study using astronaut whole blood from male and female crew members reported that the LEO spaceflight environment impacts cellular stress response pathways (including DNA repair, protein folding/degradation, and oxidative stress) in ways that could potentiate infectious disease risk for the crew. In addition, LEO spaceflight has been shown to significantly increase the virulence and stress responses of some bacterial pathogens, which in combination with altered immune responses, suggests an increased risk for infectious disease during spaceflight. Moreover, microbial monitoring of the ISS indicates the presence of opportunistic pathogens in the habitable volume. This chronic exposure to potentially pathogenic microorganisms would also be expected on LBLEO spacecraft, further indicating a need for the crew to maintain a strong immune system.

To understand the adaptation of the immune system to LEO spaceflight conditions, investigations of animals, humans and defined mammalian cell types have been conducted on the ISS and on Earth (with spaceflight analogues). The knowledge gathered on alterations in immune homeostasis resulting from the multiple stressors in space and the new mechanistic insights provided by spaceflight analogue investigations on Earth are prerequisites for defining appropriate, safe and efficient countermeasures to mitigate immune related health problems, especially in light of planned manned deep space/LBLEO exploration class missions.

Model organisms. In addition to LBLEO spaceflight immune system experiments using astronauts as test subjects, the use of human surrogate model systems is also critical for experiments that are not suitable or practical for the crew. These model surrogate systems should be readily capable of building upon previous spaceflight data and be able to improve our knowledge for crew immune system health and disease. Given that spaceflight resources are limited, having the option to use small biologically and genetically well-characterized immune and redox model systems to study the transition between homeostasis and disease are extremely useful. These models include defined human and animal cell types (including those used for development of 3-D cell cultures), and vertebrate/invertebrate animal models like *Drosophila, C. elegans,* fish, amphibians and rodents. Since these model systems have significant homology to the human disease database and have previously flown in LEO, there is existing data for comparative studies in LBLEO. In addition, some of these models have been used in LEO spaceflight missions to study the host-pathogen interaction and/or subsequent immune responses to pathogen challenge (human cells, *C. elegans* and *Drosophila*), as well as the testing of infectious disease countermeasures (*C. elegans*). Such model systems allow the use of relatively large statistical sample sizes to be monitored for multigenerational growth, evolutionary studies, population genetics, or comparative aging studies, while generally using limited spaceflight resources (power, volume, mass, etc). In particular, experiments using cell culture models and invertebrate animal models 1) have the advantage of being easy to genetically manipulate (readily allowing construction of mutants), 2) can be automated, thus requiring no crew interaction, and 3) can be accomplished even in the absence of sample return if that is a limitation in early LBLEO missions.

SPECIFIC SCIENCE

1. Determine the impact of short and long duration LBLEO spaceflight missions on cellular, mucosal, and humoral immunity, including:
    a. Global immune cell dynamics, including alterations in abundance/quantity, function/activation, and development of immune cell types (including T cells, B cells, NK cells, neutrophils, monocytes, macrophages and dendritic cells)
    b. Gene and protein expression and metabolic profiling (including *–omics*), and function of innate, mucosal, adaptive and progenitor immune cells
    c. Acute and chronic inflammatory responses, including alterations in cytokines
    d. Antibody production, including ability to confer an efficacious and protective immune response
    e. Measure the chemokines and lymphokines from a drop of blood throughout the duration of the flight.

2. Correlate the biological impact of potential LBLEO spaceflight-associated immune system dysregulation to known clinical conditions/disease
3. Understand the physical and biological causative factors of LBLEO-induced alterations in immune responses, and their interconnections, on the immune system (innate, cellular, mucosal and humoral), neuroendocrine (including sex hormones), cell signaling pathways, gene expression, cytokines, microbiome, metabolome, etc, as they relate to normal immune homeostasis or transition to disease.
    a. Determination of molecular, cellular and biomechanical/physical regulators of spaceflight-induced alterations in immune cells
    b. Determinations as to whether LBLEO-induced changes are a direct (gravity-sensing) or indirect (ex. fluid shear, mass transfer, hydrostatic pressure) effect.
4. Use of *in vivo* animal models in combination with *in vitro* cell culture models (including 3-D cell cultures) to obtain mechanistic understanding of LBLEO-induced immune based alterations and associated disease processes. While use of vertebrate and invertebrate animal models provides valuable insight into disease, they may not directly reflect the human disease process, have high variability, and their inherent complexity makes it difficult to identify underlying mechanisms. Tissue culture models, owing to their reduced complexity, are well-suited for use in evaluating molecular mechanisms that underlie the disease process that can subsequently be tested in animals to establish the relevance of the data to pathogenesis and disease.
    a. Use of knockout immunodeficient models (including vertebrate and invertebrate animals, mammalian cell culture/3-D cell culture, etc) and humanized mice to dissect specific pathways to understand mechanisms of LBLEO spaceflight-induced alterations in immune function.
5. Determine the effect of sex/gender differences on immune function in the crew during LBLEO missions (infectious disease risks, cancer risks, and autoimmune disorders). Since sex hormones influence microbiota composition, microbial virulence and immune responses, physiological differences between males and females and susceptibility to infectious and non-infectious disease is important to consider.
6. Use of longitudinal metagenomic sequencing/screening of both the crew genome and the crew microbiome to identify key biomarkers to evaluate changes over time and correlate with immune status and disease risk. This will allow correlation between alterations in immune parameters, microbial changes, and other physiological indicators that will lead to a personalized medicine approach. Profiling at crew annual medical exams (AMEs), in-flight, and post-flight.
7. Determine if menstrual cycles and oral contraception impact immune parameters of female crew. (50% of NASA's latest astronaut selection group are women).
8. Determine the relationship between immunity and radiation, including the impact of low dose, long duration radiation exposure on the immune system.
9. Determine the interconnection between LBLEO stressors, the neurological system, the immune system, and other aspects of human physiology.
    a. Determine the interconnection between oxidative stress, radiation exposure and immune function. Evaluate use of countermeasures, including probiotics and antioxidants.
    b. Determine the contribution of peripheral monocyte infiltration to the brain with regard to neuroinflammation and microglia-associated functions.

10. Determine if the increases that have been repeatedly observed in latent viral reactivations during LEO spaceflight are observed in LBLEO deep space, and if so, are they accompanied by clinical symptoms of reactivation? Does this contribute to cancer development?
11. Characterization of nutrient absorption, dietary intake and metabolism in LBLEO on immune function.
12. Development of appropriate ground-based LBLEO analogues for studies to tease out underlying mechanisms (both physical and biological) that are responsible for LBLEO-induced alterations in immune responses.
13. Determine the impact of immune dysregulation and microbiota composition in crew wound healing during LBLEO deep space missions.
14. Need for long-term (lifetime) follow-up of astronauts post-flight to monitor for LBLEO-induced alterations in immune function and adverse health events that may take years to manifest. This is especially important given the small "*n*". Studies involving the effects of spaceflight on immunity to date have been limited by a) the use of mainly pre- and post-flight samples, b) inconsistency in the duration of spaceflight, and c) use of a small number of data points. Accordingly, there are comparatively few studies that have utilized samples acquired during spaceflight, creating many inconsistencies within the current scientific literature. Further investigation is necessary to better understand the mechanisms of spaceflight effects on the immune system induced by spaceflight in order to develop countermeasures to reduce infectious disease risks for the crew.
15. Understand the impact of current preventative measures on the risk for immune-related disease events. *Ex.*, consider the consequences if there was no monitoring of environmental microbial content in spacecraft or preflight health stabilization quarantine of crew.

GOAL 4— AN INTEGRATED APPROACH TO UNDERSTAND THE IMPACT OF DEEP SPACE MISSIONS ON MUSCLE AND SKELETAL SYSTEM FUNCTION

Both muscle and bone exist in steady states. Muscle mass/quality, and bone mass/quality, are affected by the respective turnover of each tissue. Furthermore they are coupled. Muscle contraction applies stress to bones, and bones respond via physical-chemical signals by producing bone mineral. The details whereby this happens have been characterized in great depth. The lack of normal forces in spaceflight in and beyond LEO results in immensely reduced stress on both muscle and bone and departure from their steady states. The issues of bone and muscle loss are common to all long duration missions, whether or not they involve orbits beyond LEO. Astronaut risks are amplified when they are called upon to work and move on a planetary surface and are subject to an increased probability of falling, even in reduced gravity. The extent to which this condition constitutes a show-stopper in exploration beyond LEO is the subject of this research goal.

BACKGROUND

Muscle plasticity and remodeling

Bone and muscle have in common that they are always being remodeled in a not-always balanced state between construction and destruction. Interactions between anabolism and catabolism and intracellular signaling cross-talk form the basis for the balance between degradation and synthesis. Muscle plasticity, as in adaptation to exercise, is facilitated by a switch between oxidative, slow-twitch and glycolytic, fast-twitch muscle fibers, depending on the nature of the exercise.

Degradation, autophagy and muscle protein breakdown are enhanced by disuse. Initially proteolytic systems are activated, and contractile proteins and organelles are removed, resulting in the shrinkage of muscle fibers, myonuclear apoptosis and the release of myofibrilar protein. This is due in a large degree to the activation of the ATP-dependent ubiquitin-proteasome proteolysis pathway.

Muscle protein synthesis is the driving force behind adaptive responses to exercise and represents a means to measure chronic efficacy of acute interventions, including exercise and nutrition. Hormones, especially insulin and testosterone, have important roles as regulators of muscle protein synthesis and muscle hypertrophy. As a consequence of exercise disruption to muscle cell organelles activates the self-renewing satellite cells found between the basal lamina and the sarcolemma to proliferate and fuse with each other and the muscles fibers. Due to the resulting additional nuclei muscle fibers synthesize more proteins and create more actin and myosin. Higher numbers of satellite cells are found associated with slow-twitch (endurance-trained, oxidative) muscle fibers than with fast-twitch (resistance-trained, glycolytic) muscle fibers within the same muscle.

Muscle-derived signaling

Myokines, hormones and other peptides influence muscle development. Hepatocyte growth factor (HGF) is an active factor in damaged muscle recovery, and fibroblast growth factor (FGF) is important in muscle repair following exercise and may contribute to revascularization during muscle regeneration. Insulin-like growth factor-I and –II (IGFs) play a role in regulating muscle mass via gene expression and promoting muscle cell repair. Insulin stimulates muscle growth by enhancing protein synthesis by facilitating the entry of glucose into cells, especially satellite cells, not to mention availability of glucose for intramuscular energy needs. Exercise stimulates the release of growth hormone (GH) from the anterior pituitary gland in proportion to exercise intensity. GH induces fat metabolism for energy for muscle growth and stimulates amino acid uptake into skeletal muscle protein. Testosterone can stimulate growth hormone responses in the pituitary, which enhances cellular amino acid uptake and protein synthesis in skeletal muscle. In turn, muscles reacting to positive and negative stimuli produce myokines, auto-, para- and endocrine mediators actually produced and released by skeletal muscle with far-reaching effects on non-muscle tissue, providing molecular links between muscle function and whole body physiology. In endurance-trained muscle at least four transcription factors control the transcription rate of the peroxisome-proliferator activated receptor γ coactivator 1α (PGC-1α), which is considered to play the central role in the slow-twitch gene-expression program and is

recruited to more than 7500 transcription sites in the mouse genome and induces and inhibits the transcription of some 984 and 727 genes, respectively, in muscle and could be considered a therapeutic target [Schnyder, 2015]. The benefits of endurance training on the whole body are beginning to be understood based on of myokine secretion by skeletal muscle, which can be classified in some views as an endocrine organ. While myostatin serves an autocrine function in limiting muscle mass development, decorin serves a paracrine function by counteracting myostatin from any source. Skeletal muscle fibers express and release IL-6, a well-known inflammatory cytokine, and IL-8, which could play a role in neovascularization, during and after exercise. Likewise IL-15 is secreted and thought to serve an endocrine function in controlling lipogenesis. At least nine additional active peptides secreted by skeletal muscle, especially during exrecise, have been characterized, and these include two neurotrophins and the familiar growth factors VEGF and FGF-21. All of these interactions are relevant to the counteraction of disuse-induced catabolic effects.

Vesicular fragments. Sustained muscle responsiveness is dependent on the health of the sarcoplasmic reticulum (SR), the membrane-enclosed system that is responsible for the storage and release of calcium ions to sarcomeres to activate troponin C and facilitate actin and myosin fibers to interact. The actions of the SR can be studied in vitro by forming vesicular fragments of the SR membrane and measuring ion transport and other functions under environmental influences such as muscle-affecting drugs. The principal protein (80%) in the SR membrane is Ca^{++}-transport ATPase, which re-fills the SR vesicles. For example, the benzophenanthrine alkaloid, sanguinarine has been used to open Ca^{++}-release channels and induce muscle contraction. Opposing drugs, known as calcium-channel blockers, operate on the SR Ca^{++} channel protein to inhibit muscle contraction. There is a release of signal-protein-containing vesicles from normal human myotubes after rising intracellular Ca^{2+}.

Known effects of spaceflight

Time course. Similar levels of soleus muscle atrophy occur in mice at 12 days, rats at 14 days and humans at 17 days of orbital space flight (space shuttle flight STS-108). The steady loss of muscle mass with time on orbit, along with a transformation from slow fiber types to fast twitch fibers, has been documented. The threat to astronaut health and safety becomes ever more serious with increased mission duration, and has led to the development of numerous countermeasures, many of which are implemented on the ISS. Nutrition, drugs and exercise are partially effective in limiting the musculoskeletal losses in space, but they have their own limitations and are not fully effective. The threat of endangerment due to injuries resulting from weakened muscles (falls, strains, lifting accidents, etc.) has been treated as real for returning crew members in terms of cautious return to resistive activities. Significant lesions have been observed in the muscles of returning rodents after sufficient reambulation, including lesioned sarcomeres, myofibrillar disruptions, edema, and evidence of macrophage activation and monocyte infiltration (known markers of injury-repair processes in the muscle) within target myofibers [Riley, 1995]. Thus one may infer that there is muscle injury due to the atrophic process and a potential for injury if stressful stimuli are imposed on the muscle system before it can regain its proper structural and functional capability.

Morphology and contractile characteristics. In laboratory animals histochemical and immunohistochemical analyses show a reduction in the diameter of the affected myofibers of which the individual muscles are composed. The slow type of fiber is more sensitive than the faster types of fiber, which is consistent with the above observation of shift to fast-fiber prevalance. The larger fibers, whether fast or slow, are more sensitive to the unloading stimulus than their smaller counterparts [Roy, 1996]. Knee extensor and knee flexor strength losses in long-duration crewmembers after flights aboard Mir and ISS were ~23% and ~25%, respectively indicating that strength losses in the quadriceps and hamstring muscle groups were significant and similar for NASA-Mir and early ISS missions [Lee, 2004].

Force-velocity (F-V) studies conducted on the rodent soleus muscle, in which slow-twitch myofibers predominate, showed that the maximal *strength* of the muscle *was reduced* by 24% after a 6-day flight and 37% after a 14-day flight, consistent with the degree of atrophy observed at both the gross and single-myofiber level as well as a switch to a faster contractile phenotype. Indeed, maximal shortening *velocities* were *increased* by 14% and 24% in the 6-day and 14-day spaceflight animals, respectively, attributed, in part, to the de novo expression of the fast type IIx MHC in many of the slow muscle fibers. Both work- and power-generating capacities of the flight-induced atrophied muscles were significantly decreased, as was the resistance to fatigue and the ability to sustain work and power output in response to repetitive contraction [Caiazzo, 1996].

Muscle protein synthesis rate is depressed during disuse via a group of signaling pathways involving Akt, mTOR, p70S6 kinase, and 4E-BP1 all indicating that gene expression levels play an important role in the relationship between activity and myofibril maintenance, consistent with the previous statement that nuclei play a role in conditioning.

Muscle Protein degradation is stimulated by secreted factors including glucocorticoid hormones, myostatin, NFkappaB and reactive oxygen species (ROS). The heightened ROS observed in animal tissues in simulated or real microgravity may be a consequence of these pathways.

Countermeasures challenges. Deterioration of the musculoskeletal system must be prevented or a mission to Mars (and back) will not be successful. Highly refined exercise protocols and robust exercise equipment and methods to monitor functional capacity are mandatory for mitigation of the risks inherent in long-duration exposure of humans to microgravity. A huge challenge will be to provide the above within the current design of the crew exploration vehicle (CEV), which provides trivial space for equipment and crew. The cramped confines will afford little room for stretching or exercise. Modest or no power for equipment and a human life support system whose design may be marginal to support a full complement of crew and their necessary routine exercises.

Bone Mineral Loss

Loss of bone mass and size, and the accompanying increase of fracture risk, has been a major concern for long duration space flight since the outset of the space age. Bone loss has been extensively documented, beginning with the human testing on the Skylab missions, and

augmented by numerous animal studies on Russian and American space stations. The underlying mechanisms by which calcium balance is upset and bones become weaker has been adequately reviewed in the latest NASA HRP Evidence books and reports: *Risk of Bone Fracture Due to Spaceflight-Induced Changes to Bone, Risk of Early Onset Osteoporosis Due to Space Flight* and *Risk of Renal Stone Formation* (Sibonga, 2017a, b, c).

Bone breakdown occurs all the time, is part of the ubiquitous bonen plasticity and remodeling process, and is carried out by osteoclasts. It is overbalanced by bone synthesis during body growth and fracture repair, is balanced by bone synthesis during bone maintenance, and overbalances bone synthesis during aging and disuse, such as immobilization within a cast and unweighting during space flight. During the unloading of weight-bearing bones under spaceflight conditions, mineral loss may also occur in bones not normally considered to be load-bearing. After middle age in humans osteoclasts continue to be more active than bone-forming osteoblasts, slowly dissolving bone mineral and releasing the calcium and phosphate to the circulation. Osteoclasts are activated by the cytokine RANKL and, more importantly, inhibited by the natural receptor osteoprotegerin, a member of the TNF receptor superfamily.

Bone synthesis is understood on the basis of a rich research history, some of which includes fundamental gravitational physiology. Mineral bone is calcium apatite laid down by mineralizing osteocytes, which embed themselves in the mineral matrix and appear to respond to canalicular fluid motion within the lacunocanalicular space of the mineral matrix by causing calcium phosphate to precipitate at the mineralizing osteocyte periphery. This process is responsive to a wide variety of hormones and cytokines, especially including parathyroid hormone (PTH). The precursor cells of osteocytes are osteoblasts, which are the primary responders to calls for new bone and have therefore been subject to intense study in vitro. They pass through several stages of differentiation ending in mineralizing osteocytes which then become terminally differentiated, embedded, responsive osteocytes in the mineral matrix. Osteocytes also secrete sclerostin, which inhibits osteoblasts (built-in negative feedback loop). These steps are all potential targets for bio-countermeasures and still rich in possibilities to characterize epigenetics and metabolomics.

Bone-derived signaling is relevant to the whole-body response to the spaceflight environment. Osteocytes play the key role in mechanosensing and mechanotransduction in bone and regulate the function of both osteoblasts and osteoclasts [Dallas, 2013]. Bone anabolic therapeutics may therefore be drugs that mimic, to the osteoblast, the effects of mechanical loading thereby leading to more nearly normal calcium and phosphate homeostasis. As bone formation declines, what messages are sent to other parts of the body? Bone, and osteocytes in particular, can be considered an endocrine system in that signaling molecules such as FGF23 (master regulator of serum phosphate and calcium homeostasis) and sclerostin (inhibitor of bone formation by osteoblasts), which affect distant organs or other cells, are secreted into the vascular system. Dying (apoptotic) osteoblasts are also a source of intercellular signaling. Sclerostin, osteocalcin, and ORP150 are expressed by mature osteocytes, and several differentiation and transcription factors are expressed as osteoblasts move along their differentiation pathway.

Time course of known spaceflight effects. Measureable bone loss occurs after as little as two weeks on orbit in humans and within about 12 days in mice. In mice undergoing hindlimb suspension the loss follows a similar timeframe. The rate of bone loss in the proximal femur seems to be variable in crew members, with some losing up to 20% of bone mineral density at some sites within a 6-month period and others losing less than 1% per month. In some individuals only some areas of the lower extremities are affected. In this and other examples an emerging principle is the use of genotyping in crew selection. This is a controversial ethical subject that should not be ignored simply because it is controversial. In situ stiffness measurements are useful, and non-invasive methods are available that exploit data-rich signals from diagnostic ultrasound, miniaturizable, versatile instrumentation potentiallyi suitable for the baggage of the traveler beyond LEO. In any case the rate of mineral loss is astonishing relative to most terrestrial pathological conditions. A crew member loses as much lower-body bone mineral in 1 month as a postmenopausal woman in 1 year.

Bone quantity/Bone quality in spaceflight. Cortical and trabecular bone respond differently to weightlessness and radiation. Bone density measurements can now be performed on rodents on the ISS; however, it is difficult, if not impossible, to measure trabecular and cortical bone loss independently on orbit. Differential signal transduction analysis might lead to an understanding of where bone mineral is deposited.

Reduced bone synthesis. It is believed that there is reduced fluid motion within the lacunocanalicular space. Osteocytes, in their paracrine function, stop recruiting osteoblasts to differentiate into mineralizing osteocytes, presumably via the sclerostin signaling pathway.

Increased bone breakdown results in the release of calcium and phosphate into the circulation as osteoclasts carry out their normal functions while osteogenesis is fading. It is believed that this increases risk of kidney stone formation.

Unique aspects of long-duration space exploration on muscle and bone include mission duration and radiation, which has not been found, at least at high doses to contribute additional risk in potential interaction with the effects of weightlessness. Research for LBLEO missions should include advanced in-flight physiological monitoring of bone mineral density, bone dimensions, muscle strength and muscle fatigue as well as advanced biomarkers.

Fractional g. There have been no studies either in rodents or humans at fractional g (inertial accelerations between 0.0 and 9.8 ms^{-2}) owing to the total lack of facilities. At the very least, a rodent centrifuge should be implemented on ISS while this LEO platform is still available and has the capability to monitor bone density on orbit. Human testing should be possible when commercial inflatable space stations become available in LEO.

Interactions with nutrition and/or radiation. On 6-to-30-month flights beyond LEO such the bone lost as a result of reduced gravity will be augmented by radiation exposure. The last 5 years have seen enormous progress in the interest in and understanding of the effects of ionizing radiations, including heavy ions, on bone. In short, most of the damage is done to trabecular bone, and the damage is seen as loss of connectivity; that is, mineral columns in the spongy matrix become interrupted. Installing a rodent research facility on the first manned mission

beyond LEO, if only for this purpose alone, would greatly aid the understanding of what might be expected o the human musculoskeletal system.

Sensorimotor integration undergoes adjustment during human space travel, mainly adjustment to spatial orientation and postural control in an altered gravity environment. Early on Soviet cosmonauts and American astronauts experienced bouts of "space motion sickness" (SMS) shortly after entering weightlessness and lasting as long as several days. With symptoms resembling motion sickness, such as sea, air or car sickness, this phenomenon was both dangerous and demoralizing. To avoid an implication that the disability was a mark of weakness, it was earlier referred to euphemistically as "Space Adaptation Syndrome" (SAS). It is now dealt with both by limiting head movements early in space flight and by the use of medications, chiefly intramuscular injections of promethazine or prophylactic use of scopolamine or promethazine. In general the symptoms are reduced on repeat flights, possibly because astronauts learn to limit head movements and to retain some of the previously learned adaptation. Recurrence of symptoms can occur after landing back on earth (Earth Sickness) or potentially on a planet with a different gravity level, although it was not reported during the Apollo lunar exploration missions. In any case astronauts are likely to experience some spatial disorientation (SD) in orbit and on a different planet. An extreme SD example is the "inversion illusion", in which the weightless astronaut may feel "upside down" and eventually accept the spacecraft floor as the "down" direction. The explanation for SD lies in the function of the vestibular system in altered gravity. On Earth, each head movement about an Earth horizontal axis (normally pitch or roll) produces compatible responses of the linear accelerometer in the inner ear (the otolith organs) and the angular rate sensors (the semicircular canals). A rolling movement of the head from the upright toward the left shoulder, for example, produces responses from the hair cells in the vertical semicircular canals which register the angular movement correctly. Furthermore, the head orientation relative to the vertical is nearly correctly measured by the responses of the otolith organs, stimulated by the component of gravity pulling the seismic mass (the otoconial membrane) of the otoliths toward the left. The two signals are compatible. However, if the subject is accelerated, toward the right for example, the otoliths will respond similarly, as if the head were tilted to the left. This "otolith instability" is normally taken into account as we move about on the surface of the Earth. In weightlessness, however, the otoliths organs no longer respond to steady tilt since they are not being stimulated by any net inertial force (gravity minus acceleration). The semicircular canals, meanwhile, continue to correctly signal angular pitch and roll. It is the *conflict* between the sensors which is thought to be the basis for motion sickness, whether in space or at sea. With repeated exposure to head movements, especially in the presence of visual cues, SD is overcome and motion sickness is diminished – at least until transition back to another G level. The process, known as the Otolith Tilt-Translation Response (OTTR) hypothesis, is thought to explain the adaptive capability to sensorimotor integration – on Earth, in hypergravity on a centrifuge, or in hypo-gravity on the moon or in parabolic flight [Young, 1984; Young et al., 1984; Reschke et al., 1994].

The adaptation to altered gravity in long duration missions beyond LEO is expected to resemble that seen in long duration missions in LEO with the exception of possible Coriolis forces and cross coupled accelerations, to be expected during head movements while undergoing artificial

gravity (AG) centrifugation, little difference is to be expected. There is no reason to think that the adaptation of sensori-motor integration will be influenced by radiation or other environmental aspects of LBLEO. Whenever a change in inertial force is experienced, on a centrifuge or on another planet, for example, a certain amount of exposure and adaptation is to be expected. The transition could be accelerated by gradual incremental exposure to the new stimulus. However, based on experience to date, the adaptive processes can be expected to proceed in the direction of adequate performance and behavior in the new environment.

Potential models (strengths and limitations)

In vitro cell research has revealed tremendous detail concerning signal transduction pathways in osteoblasts differentiating into osteocytes. Osteoblasts have been the traditional favorite for in vitro study owing to their ready availability, and cultures have been used in spaceflight experiments. Osteoclast cultures have served extremely useful purposes in understanding bone resorption (including the function of osteoprotegerin), and these are readily prepared from bone marrow aspirates. They are derived from the monocyte/macrophage lineage of the bone marrow, and they are thought to be responsible for maintaining marrow space for hematopoietic functions. Studying cell cultures in space flight can achieve only limited objectives owing to the significant difference between in vitro and in vivo transport phenomena.

Whole organism research using, for example, knock-in mice with GFP-fusion proteins are a powerful tool for tracking the expression of genes. Rodent hindlimb suspension models have become somewhat of a gold standard for disuse atrophy studies in pharmacological research. Muscle and bone loss patterns in mice are sufficiently similar to those experienced by crew members on orbit to allow, at least, utilization of mouse genetics, epigenetics and metabolomics to help understand, if not extrapolate directly, predictions of the effects of countermeasures applied to humans traveling beyond LEO.

Research opportunities. The control of the dynamics of bone and muscle regeneration, continued utilization of laboratory-animal unloading, the use of intermediate-g via ISS centrifuges, and studies of natural disuse as in hibernation and estivation are examples of opportunities to understand space-based musculoskeletal deterioration and to move toward more "natural" countermeasures.

Potential for translation to humans. Most rodent-research findings, especially those related to countermeasures should be indirectly (via signal transduction interpretations) or directly (via scaled data extrapolation) applicable to human crew members. These include the results of rodent studies that demonstrate musculo-skeletal countermeasures and separate muscle and skeletal countermeasures, many of which have been performed in corporate pharmaceutical laboratories. Differential responses to a fractional gravity environment can, and should, be studied in the near future utilizing partial-gravity centrifuge facilities on ISS. Nutraceutical studies have also been performed using rodents, and a wide variety of them (some of them delicious) has been found to fight oxidative stress and boost mineral metabolism. An appropriate nutraceutical approach could avert the dangers of strong drugs and replace potential dietary insufficiencies.

Pharmacological countermeasures. Numerous drugs have been developed to counteract the loss of muscle and bone in diseased states and in microgravity. Some of these have been tested in rodents in space flight. A recent example is the testing of myostatin inhibition to prevent skeletal muscle atrophy and weakness in mice exposed to long-duration spaceflight by Eli Lilly Co. using the NASA Rodent Research-3 mission on ISS in 2016. Over the years pharmaceutical countermeasures have involved the testing of osteoprotegerin (2001), myostatin inhibitor (2007), sclerostin antibody (2011) in rodents in space flight as well as in the hindlimb suspension models.

Physical countermeasures. It is widely held in the orthopedic world that impact events are essential to the maintenance and repair of weight-bearing bones. This observation would be consistent with a proposed role of stress-generated potentials via fluid electrokinetics in osteogenesis. Hypothesis-driven studies of intermittent impact stress and/or electrostimulation as countermeasures to disuse atrophy need to continue. Electrostimulation alone can increase muscular strength and tolerance to static loads; however, some form of exercise, presumably aerobic, is required for cardiovascular maintenance. One issue of exploration beyond LEO is the size and mass of exercise equipment and its impact on orbital transfer and propulsion requirements. Resistance training can be mimicked by "Hybrid training" (HTS), in which electrostimulation signals are generated by muscle contractions that cause opposing muscles to contract. The HTS would be ideal for the smaller spacecraft envisioned for manned exploration missions beyond LEO and deserves intensified attention [Shiba, 2015]. A leading candidate for an extended mission countermeasure is Artificial Gravity (AG)– either continuous or intermittent rotation of the entire spacecraft or of an on-board centrifuge. The key parameters of AG are the rotation rate, g-level, exposure duration and radius. Both animal centrifuges and human short radius centrifuges may be employed, on the ISS or independently, to determine the desired size and spin rate. An animal centrifuge capable of spinning rodents for long duration will be available on the ISS and should be provided for further testing in anticipation of LBLEO missions.

SPECIFIC SCIENCE

1. Differentiate between loaded and unloaded muscle in the flight environment (locomotor muscles vs. diaphragm)
2. Describe the effect of the fractional g environment on bone and muscle independently.
3. Establish a nominal translational framework from cell culture to human
4. Genomic and proteomic profiling of muscles and bones to establish time-dependent aspects of the expressome (transcriptome and proteome). What is the asymptote?
5. Muscle-derived peptide, exosome and microvesicle effects on other tissues, notably heart
6. Optimized countermeasure strategy incorporating nutraceutical, pharmacological and physical countermeasures. Multidimensional optimization for bone and muscle independently; then seek points of overlap.
7. Establish efficacy of biomimetic models of hypokinesia and loading with translational potential to long-duration spaceflight.

8. Interactions between anabolism and catabolism; Intracellular signaling cross-talk affecting net muscle protein accretion or loss
9. Signaling properties that determine where bone mineral is deposited
10. Role of the ubiquitin-proteasome and the autophagy-lysosome systems in spaceflight-muscle atrophy
11. Muscle protein synthesis in long-duration spaceflight
12. Calcium isotopic analysis to assess bone mineral balance in mammals and humans during long duration spaceflight
13. Interaction of high LET radiation environment with bone formation
14. Improved ability to visualize cortical and trabecular bone in-flight

GOAL 5— AN INTEGRATED APPROACH TO UNDERSTAND THE IMPACT OF DEEP SPACE MISSIONS ON CARDIOVASCULAR SYSTEM FUNCTION

This goal seeks to address the wide array of risks to which the heart and circulatory system are susceptible in the Beyond LEO environment. From the viewpoint of cardiovascular health environments and programs beyond LEO differ from those in LEO in several ways: much longer duration, prolonged exposure to galactic cosmic rays, and loss of access to terrestrial facilities, for example. Issues addressed in this section build on these factors. NASA's well-funded Human Research Program (HRP) operating from JSC deals mainly with human subjects, funds grants (to the external science/university community) related mainly to human studies, and also deals with risk assessments for astronauts and sample and data collection. Cardiovascular changes for astronauts are a major topic within this portfolio including risk assessments, monitoring astronauts before during and after spaceflight, sample collections and data analysis. Space biology and HRP do of course work closely in collaboration, because as we have seen historically we would have no "'omics" or genetics or molecular biology or stem cell biology today without bacterial, plant, fruit fly, rodent and other model organism genetics and biology.

BACKGROUND

Quoting from the *NASA Space Life Science Plan 2016-2025, "*In 2011, the Committee for the Decadal Survey on Biological and Physical Sciences in Space of the National Research Council published its decadal survey recommendations to NASA, 'Recapturing a Future for Space Exploration: Life and Physical Sciences Research for a New Era' that established guidelines for NASA's approach to conducting research in the Space Life Sciences. Major recommendations of the Decadal Survey included" the following research items relevant to cardiovascular research in and for space exploration (quoted here in part, skipping items 1, 3, and 5):
"2) Cell and Molecular Biology studies using state-of-the-art cell biology tools to monitor evolution of genomic changes in microbes, plants, animals or other biological systems in spaceflight;
4) Animal and human studies to evaluate physiological mechanisms of bone, muscle, cardiopulmonary, immune, and neural functions during adaptation to spaceflight;

6) Cross-cutting studies, including artificial/fractional gravity, radiation, and gender differences; 7) Activities facilitating open public and scientist access to the products of NASA Space Biology research data and results by building data archives and data management tools, especially in the area of systems biology (genomic and other "omic" experiments)."

The text that follows emphasizes these recommendations in more specific terms as they relate to cardiovascular issues, namely arrhythmia, vascular maintenance and development, instrumentation, surrogate model organisms for molecular studies, 'omic studies on humans, partial gravity and accessing human data. The historic paradigm of research that directly enables exploration or would produce fundamental new knowledge is as follows.

Historically a robust cardiovascular system evolved in the animal kingdom to maintain an appropriate blood supply and pressure in the various organs. "Understanding how these physiological systems sense, adapt and respond to gravity cannot be fully achieved on the ground; it requires the use of spaceflight, i.e., the use of microgravity as an investigative tool. Just as one needs to examine the entire light spectrum in order to determine the capabilities and mechanisms of the visual organs, so too must we utilize the complete gravity spectrum, from hypo-gravity to hyper-gravity, to understand how gravity influences life across the gravity continuum, i.e., both on and off the Earth." [NASA, 2016]

Cerebral blood flow: There is a need to understand cerebral blood flow and vascular resistance in space flight. Evidence is accumulating that human vision is degraded by long-duration space flight that may be associated with vascular factors. Human headward fluid shifts in microgravity are well documented but the responsiveness of the vascular system is not well understood. Studies on the basilar arteries of mice on three Shuttle missions and the 30-day Bion M1 mission showed that the physical attributes of the arteries were not different between experimental groups but there was clear microgravity-related attenuation of both vasoconstrictor and vasodilator properties that could limit the range of vascular control of cerebral perfusion and impair the distribution of brain blood flow during periods of stress [Sofronova, 2015: PMID 25593287]. The short duration of these exposures should be noted. This result needs to be further studied in animals with the goal of translating the results to humans and clarifying the potential impact on related chronic vision problems associated with long-duration spaceflight.

Arrhythmia: Atrial and ventricular premature contractions, short-duration atrial fibrillation, and non-sustained ventricular tachycardia were reported by previous spaceflight programs. Arrhythmias during spaceflight are related with hypokalemia, microgravity, changes in the autonomic nervous system, and physical stress. During Apollo, SkyLab, and Space Shuttle EVAs dysrhythmias were recorded, such as VPCs, APCs and multifocal VPCs. Electrocardiograms are always monitored during EVAs. There have been a total of 75 arrhythmias and 23 conduction disorders reported by the Russian medical community to NASA. The second most frequent medical problem during the MIR program era was arrhythmias. An Institute of Medicine review has commented on the veracity of findings concerning arrhythmias [National Academy of Medicine, 2016]. Electrolyte imbalance has been implicated, especially potassium insufficiency [Anzai, 2014].

Surgery: The circulatory system plays a critical role during any surgery. The escape of blood and body fluids during surgery in low gravity presents unique problems. Inventive approaches, such as the aqueous immersion surgical system [Hayden, 2015] will need to be explored and practiced. Surrogate animals for surgical practice are typically pigs, dogs and occasionally rabbits, models not previously considered for research IN space but nevertheless studied on low-gravity aircraft, especially for cardiac surgery procedures.

Vascular maintenance and development: Is g loading necessary for normal development of the cardiac system? A small amount of research has been performed using the hind-limb suspension model [Vener, 2004] in which cardiac atrophy and myocyte apoptosis was reported after 14 days of hindlimb unloading in Rats.

Cardiovascular damage: The possibility of long-term degenerative effects of deep space travel on cardiovascular function has not been well described or substantiated. In July 2016 news went out all over that space crew epidemiology revealed evidence of premature deaths due to heart disease in astronauts. Cited, for example, was James Irwin suffering his first heart attack at age 43. While there were no significant differences in heart-related mortality rates between non-flying and LEO astronauts, the heart-related mortality rate among Apollo lunar astronauts (43%) was 4 to 5 times that of these groups. Flights beyond the Earth's magnetosphere distinguished the Apollo crew members from all other astronauts [Delp, 2016]; however, with such a small sample size a possible role of small differences in radiation quality, dose and dose rate can be ruled out as a causative factor.

Ionizing radiation: The radiation environment in deep space is significantly different from what it is in LEO. The fluence and complexity of the heavily ionizing deep space radiation is impossible to mirror accurately on the ground-based facilities (such as Brookhaven National Lab or BNL etc). These particle types are also exceedingly damaging to biological tissue. As we cannot do any radiation testing with humans, we need to use a combination of human data gathered from low earth orbit, radiation studies with mammalian models at facilities like BNL, combined with deep space radiation with surrogate biological systems and come up with predictions of how we think humans will fare in deep space over long duration missions in this damaging radiation environment. Very high whole-body doses (1.0 Gy) of simulated cosmic particles to mice have been shown to produce "impaired endothelium-dependent vasodilation through the NO signaling mechanism apparently mediated primarily through greater NO scavenging by reactive oxygen species, as evidenced by higher vascular protein content and activity of xanthine oxidase in peripheral and coronary arteries" [Delp 2016]. At lower doses (0.15 Gy) cardiac function "significantly declined in ^{56}Fe ion-irradiated mice at 1 and 3 months but recovered at 10 months, and ^{56}Fe ion-irradiation led to poorer cardiac function and more adverse remodeling and was associated with decreased angiogenesis and pro-survival factors in cardiac tissues at any time point examined up to 10 months" [Yan, 2014]. Further studies of these effects are underway.

Model research organisms: Mechanistic information has been gathered in recent years about cardiovascular function using a simple model such as the fruit fly [Bier, 2004]. The fruit fly has been used to understand human cardiac function at the level of gene function. For example KCNQ potassium channel mutations cause cardiac arrhythmias in *Drosophila* that mimic the

effects of aging [Ocorr, 2007]. Diabetic cardiomyopathy, mechanical regulation of cardiac aging, and signal-transduction-related preservation of cardiac contractile function are discoveries that have been facilitated by advancing technology [Ugur, 2016] that has allowed the tiny fly heart to be studied in detail that has shown striking similarity to the human heart both genetically and functionally. A recent fruit fly experiment on SpaceX-3 revealed substantial changes in cardiac structure and function in animals bred entirely in space and analyzed after return to the ground. The fruit fly heart is now being exploited for large scale testing of human cardiac disease variants for identifying new genetic (and epigenetic) risk factors of human heart disease. A few important examples include (a) "52 Genetic Loci Influencing Myocardial Mass" (van der Harst et al. 2016) (b) "A global in vivo *Drosophila* RNAi screen identifies NOT3 as a conserved regulator of heart function". (Neely et al. 2010). This research demonstrates how comparative genomics between *Drosophila* and humans are helping identify new genes that are important for human cardiac function and may act as targets for novel therapeutics.

Enabling technologies. For cardiovascular and related research IN space, vertebrate model organisms need to be similar to model organisms used on Earth (pigs and dogs) to include relevant baseline data. Automation is essential. We need more information from spaceflight for future DEEP SPACE missions. For the next several years, while people prepare for human missions to Mars, we will only have the capability to use small fully automated payloads to do the preliminary science to prepare for long term human exploration in deep space. In many cases we will not have sample return (e.g. small payloads piggy-backing as secondaries that are sent into solar orbit at one astronomical unit) or in a few cases there is the option of sample return. Either way, these missions will all be unmanned and therefore need to be fully automated. Therefore only biological studies that can withstand some amount of pre-launch time unpowered and untended on the pad, and then fully automated after launch are feasible in this scenario. So as we wait to send humans to deep space, a two-pronged approach is needed: collect as much human data as possible from past and future low Earth orbit (LEO) missions and analyze them, but simultaneously use human surrogate systems/models in these automated payloads and gather as much information as possible to prepare for long duration exploration. Invertebrate model organisms help guide the direction for relevant and important science research that needs to be done in order to make future human exploration in deep space a reality. We need a combined approach of mining the human data that we have from low earth orbit along with targeted studies using model systems in deep space over the next several years. Saliva sampling has revealed modifications in adrenergic proteins [Mednieks, 2014] indicating that saliva sampling, which is non-invasive, may represent and opportunity for real-time monitoring and diagnostics given the appropriate laboratory capabilities aboard spacecraft beyond LEO.

Instrumentation: Cardiovascular instrumentation is advancing with breakneck speed. It is very important to be alert to new and relevant (space-adaptable) technologies, such as the recently developed Hand-held laser scanner for cardiac assessment [Biooptics World, 2017]. Biotelemetry and rapid-freeze technologies for experiments in space are also coming along, and these should be exploited to the extent possible for the monitoring of function and the preservation of both human and animal samples.

Human data availability for research: There are decades of good pre-, during- and post-flight data on humans to be made available to researchers who understand clinical cardiovascular

medicine. Humans who participate in space flight at public expense should be required, as a condition of participation, to have their cardiovascular medical data, including spaceflight sampling, available in such a manner as to provide 'omics data to be archived in NASA's GeneLab database. From ground-based pre-flight data astronauts face the risk of medical disqualification; however, for exploration beyond LEO medical disqualification is the better part of prudence, and fully qualified humans who volunteer to be test subjects are likely to be plentiful.

SPECIFIC SCIENCE

Core questions should drive discovery research utilizing broad vision and resources for multi-use science instrumentation and results sustainable for many experiments.

1. Try to progress top down from core questions and goals and let those drive the science. Rather than continue along comfortable lines doing what we are doing and casting it in forms that seem to address the big picture, if only vaguely in some cases.
2. Determine the significance of arrhythmia. There is a need to justify its space flight relevance. Flight surgeons, high-level cardiologists and basic electrophysiologists need to collectively arrive at appropriate conclusions. There are no direct data relating significance, although arrhythmias have occurred, even on EVA.
3. Determine, on the basis of theory and experiment, the potential benefit of partial gravity produced by inertial acceleration in a rotating frame ("Artificial Gravity", AG). Cardiovascular health is but one component of such potential benefit, but the whole body depends on cardiovascular health. How much acceleration is enough? The design of human-scale centrifuges into long-term human missions needs to be taken much more seriously and given a very high priority. These may require initial testing using larger experimental animal (and plant) models. The presence of an animal centrifuge scheduled for the ISS affords an opportunity to begin in-flight physiological studies on mice – but eventually the investigations must be extended to humans on orbit.
4. Measure the occurrence of tissue damage, however defined, and its cause-and-effect relationship to cardiovascular health. Is there a long-term tissue effect, for example, due to lower-body hypotension?
5. Mechano-biology and oxidative stresses have been found to be related at a high level and need to be pursued in the context of cause-and-effect relationships. The mechanical properties of vascular tissue and the interplay of oxidative stress under prolonged spaceflight conditions could yield critical understanding of the vascular response.
6. Explore safe countermeasures against arrhythmia, blood-pressure changes and lower-body hypotension. Compare the effectiveness and crew time of current ISS exercise and Advanced Resistive Exercise Devices (AREDs) as countermeasures to alternative protocols, including electrical stimulation, gravity suits, Lower-Body Negative Pressure (LBNP) and "Artificial Gravity".
7. Hold serious discussions about mission durations and the changing demographics of humans that NASA sends on space missions. With commercial space on the rise, it is quite possible that paying customers will "beat NASA to the punch" in some cases, and there the demographic will move toward an older socioeconomic sector potentially

including those already on a path of cardiovascular compromise. This is where a new approach beyond traditional "roadmapping" as occurred before human genomics, epigenetics, and other human 'omics . The cardiovascular 'omics across all ages, races and genders will need to be considered.

8. Discern vertebrate animal models that will allow for cardiovascular and other systems to be studied. The Univ. of Arkansas mouse and rabbit heart data sets should be considered.
9. Make maximum use of three-dimensional biomimetic vascular models as a means of rapidly testing for loss of barrier function including cell migration-based mechanisms for fibrotic diseases and the roles of cytokines and factors associated with inflammation. These organs-on-a-chip are built from human cells and tissues and can be manipulated in ways that mimic spaceflight conditions and, for that matter, can be studied in space flight.
10. Make maximum use of existing biological specimens that are available, NASA Ames Life Science Data Archive (ALSDA) for example, for research and archiving. Stored biological samples can and should be analyzed for changes using the best available methods of the day (depending upon sample type, cell-free DNA, exosomes, etc.) and all future human-crewed flights include aggressive sample collection and preservation to allow for future measurement techniques to be applied downstream. As 'omics data are accrued they should be archived in GeneLab. More importantly, discern their relevance and applicability to cardiovascular conditions encountered or potentially expected in long-term space flight.
11. Maintain and study a crew member cardiovascular database and obtain access to mission medical records. There are decades of good pre-, during- and post-flight data on humans. This should be made available to researchers who understand clinical cardiovascular medicine. Recent 'omics data should be archived in NASA's GeneLab database. Humans who participate in space flight at public expense should be required, as a condition of participation, to have their cardiovascular medical data available in such a manner as well as other medical and genomic information except irrelevant/very personal items. The risk of medical disqualification is, of course, a big factor, but there are plenty of capable humans who would gladly volunteer to be test subjects. Consider missions where gathering physiologic and molecular data (not just cardiovascular) is a prime driver, not an afterthought.
12. Determine the applicability of a mouse model to query cardiovascular questions developed from astronaut data. Existing physiological data can be used for the generation of hypotheses that can be legitimately tested in gene-edited mice with signal-transductions modifications that cannot be ethically implemented in humans.
13. Take full advantage of recently-developed versatile cardiovascular instrumentation like phased-array ultrasound, in-vivo fluorescence and laser sounding devices to adapt them for long-term space missions with minimal requirements for spacecraft power, mass and volume. Serious consideration should be given to continuous (where possible) cardiovascular data collection via unencumbering wearables and, periodically, more encumbering but data rich methods such as blood draws, 12-lead ECG's, pulse wave velocity measurements, ultrasound, etc. Automation is always useful and is the trend on Earth anyway. The key is to make the automation, and hence the instruments, more generic and thus useful across wide varieties of experiments rather than "custom" for a mission experiment. It will be necessary to focus on platforms that can be built, gather

flight legacy proof, and be used for many experiments. This will require broad vision and adequate resources.

14. Utilize the tools and information gathered on molecular mechanisms learned from studies of invertebrate model organisms such as *Drosophila*. Flies have a heart that is developmentally homologous to the vertebrate (including human) heart and has therefore been used to characterize the structural, functional, developmental and genetic underpinnings of heart function and disease.
15. Effects on structure and function in wild type and sensitized mutant populations
16. Population genetics with well characterized genetic models to identify genes and gene families that play an important role in cardiac function and in adapting to the space environment
17. Is mechanical/g loading required for normal development? Looking forward 50 years, a human pregnancy in space is not out of the question. Vascular differentiation is also part of post-traumatic and/or post-surgical wound healing. A more far-out question is: Can development *in* weightlessness lead to development *for* weightlessness?
18. Review incidences of adaptation to space flight and re-adaptation to 1xg/g-load transition (including orthostatic intolerance) to assess the possibility of mission failure. There is a considerable literature on this subject, and mechanical countermeasures have been studied, but safe medical countermeasures, possibly tested in animals, need to be considered.

GOAL 6— AN INTEGRATED APPROACH TO UNDERSTAND THE IMPACT OF DEEP SPACE MISSIONS ON CENTRAL NERVOUS SYSTEM STRUCTURE, ANIMAL BEHAVIOR AND CREW PERFORMANCE AND HEALTH

This goal seeks to understand adaptive and maladaptive changes that occur to the central nervous system (CNS) of animals in response to the altered environment in space during spaceflight missions beyond low earth orbit. The space environment is characterized by a combination of micro- and hypo-gravity fields, confinement, isolation, modified circadian cues, artificial habitats in closed life support systems, and chronic exposure to space radiation. This goal further seeks to understand alterations in CNS function that could lead to impaired crew performance during spaceflight missions beyond low earth orbit and their long term health post flight.

A variety of behaviors have been shown to be altered during low earth orbit spaceflight or during the re-adaptation to ground environments in invertebrate and vertebrate models. In humans, measures of attention and cognition, as well as affect have exhibited changes and the vestibular system exhibits profound temporary disturbances during adaptation between gravity levels. Social interactions and team based activities are also prone to variation.

As compared to low Earth orbital spaceflight, missions beyond low earth orbit will be characterized by greater duration, smaller habitat volume, greater isolation and mission self-sufficiency, more restricted communication with the ground, and greater accumulated radiation dose. Depending on mission architecture, there may also be requirements for operations on planetary bodies that will occur in hypogravity fields including 0.17 and 0.38 **g**. These environmental constraints will require significant neural plasticity in order for organisms (including humans) to successfully adapt and maintain homeostasis. Successful adaptation and

plasticity must also occur in the context of spaceflight-induced changes to other body systems including sensory, musculoskeletal and immune systems.

BACKGROUND

Body designs and neural control systems have all evolved in the presence of a 1-**g** gravity field which serves as a fixed sensory and orientation reference and a mechanical constraint. The environments in which organisms live are also subject to gravity-imposed constraints and behaviors specific to land, water and air components of their habitats. When organisms are presented with novel environments they are programmed to maintain and restore homeostasis based on 1-**g** based designs and control system schema. The space environment is characterized by a combination of micro- and hypo-gravity fields, confinement, isolation, modified circadian cues, artificial habitats in closed life support systems, and chronic exposure to space radiation. Biological systems placed in space environments will attempt to adapt by using existing stereotyped procedures or by employing new compensatory formulae which may be successful or maladaptive. The critical feature of the CNS in dealing with the environment is plasticity which refers to lasting changes in neural circuits of the brain in response to experience. It is manifest at all levels of biological organization from molecular to cellular to tissue to system levels. Plasticity impacts memory, motor function, and communication and encompasses physical remodeling of synapses and pathways, molecular signaling cascades, gene expression and epigenetic modification of genes. It can be negatively impacted by a variety of stressors.

Neuroscience experiments in space have employed rodents, insects, fish, amphibians and microorganisms. They have primarily examined adaptation of behaviors after shifting between gravity levels as flight experiment durations have generally been about 2 weeks, much less than the lifespans of the animals. Many different developmental stages have been examined as well as both sexes. The greatest focus has been on the vestibular system as a direct gravity sensor and its central processing.

Human crews will have to deal with the same disturbances as animals but with a much richer behavioral repertoire and much greater cognitive abilities. They will recognize the dangers involved in missions, will have to deal with any interpersonal issues while in confinement, will be isolated from their families and feel helpless if family problems arise. They will need to perform complex tasks in a team setting, may experience physical discomfort and sleep deprivation, and may become bored without "meaningful work" during cruise phases of long missions. Prior experience shows that they will experience sleep deprivation or poor sleep quality which impairs performance. If rendezvous or landing on planetary bodies is involved there may be multiple periods of adaptation to new gravity levels. These and other issues will be dependent on mission architecture and operations.

Gravity and Spaceflight Factor Effects. There are hundreds of publications related to effects of gravity and spaceflight factors on the nervous system which is beyond the scope of this document to review. However, a number of examples will illustrate the variety of effects that need elucidation. Numerous features of spaceflight environments may modify sensory inputs to the CNS. For example, under micro-gravity, sensory frames of reference are altered as organisms are not held to surfaces, may translate and rotate freely, and will have lost the fixed "up-down" reference provided by the vestibular system and distributed proprioception systems that detect restoring forces (often reduced in intensity) from interaction of the body with surfaces

and fluids. Visual systems that have been "trained" to associate and align object shapes and orientations with vestibular-mediated signals will have to adapt to "unaligned" cues. Visual and auditory signals will have new meanings in environments in which signal origins may be anywhere in a three-dimensional environment instead of constrained to surfaces and "lower" locations. Motor programs will have to adapt to these modified sensory inputs, inertia, and gravity-dependent musculoskeletal properties, for all bodily activities, including walking, swimming, feeding, eliminating wastes, reproduction, etc. Frustrations that result from constant requirements to adapt or result in maladaptation may subsequently lead to stress with concomitant physiological changes including endocrine changes and oxidative stress.

Invertebrate animal models. Results from a variety of studies indicate that across the spectrum of animal species, gravity influences behavior and CNS function in consequential ways. The nematode model organism, *C. elegans*, was studied on Shenzhou-8 for behavior and fertility. Locomotory behavior, including speed of locomotion, frequency of reversals, and rate of body bends were found to be normal [Qiao et al. 2013]. Worms flown on STS-42/IML-1 were able to mate and reproduce for two consecutive generations on a semisolid substrate in microgravity indicating that complex controlled locomotion and mating behavior programs were stable [Nelson et al. 1994]. The mechanosensing organ in the land snail (*Helix aspersa*) responds to gravity via a dense statocyst and an epithelial layer containing sensory hair cells that outputs to the CNS and elicits compensatory body reflexes to stimuli. Snails flown for 12 or 16 days on Foton satellites and tested 13 – 42 hrs post landing responded more quickly to reorientation, were less directionally specific than controls, and upregulated a neuropeptide linked to ciliary beating in the gravity sense organ all suggesting an upregulation of sensitivity to acceleration [Balaban et al. 2011]. Young male *Drosophila* exposed to microgravity showed an acceleration in aging-like phenotypes. In a 14.5-day Space Shuttle flight (IML-2) young male flies were tested for life span and behavior. Mature animals exhibited a striking increase in locomotor activity while a smaller increase occured in recently hatched flies. Flies in microgravity walk very actively but rarely jump as in the initiation of flying. Parallel 1 x **g** centrifuge controls did not show such differences. [Benguría et al. 1996]. Crickets possess an external gravity sensory structure which is stimulated by postural displacements of the animal and induces a compensatory head response. The position sensitive interneuron, PSI, transfers information from this sense organ to the central nervous system. Experiments on Neurolab [Hom et al. 2002] showed a significant PSI-mediated response to micro- and hypergravity and levels of a specific neuropeptide were elevated at 0 x **g** versus 1 x **g**. Other flight experiments have shown alterations in locomotor behavior of wasps, bees, moths and insects from other orders. Orb weaver spiders were flown on the STS-126 mission to the International Space Station, and they exhibited good viability but their webs were of a chaotic form suggesting that a gravity reference cue may be needed for proper web building [Space.com 2012].

Fish in microgravity. In vertebrates altered gravitational environments can induce malfunctions of the inner ears, based on irregular movements of the semicircular cristae or on dislocations of the inner ear otoliths from the corresponding sensory epithelia. This produces illusions of tilting which do not match inputs from other sensory organs (especially the visual system) and results in sensory conflicts. In humans, the sensory conflicts may lead to space motion sickness. Fish model systems have been used frequently to explore interactions between visual and vestibular cues with the dorsal light response (DLR) [Rahmann and Anken, 2000]. It is elicited by side illumination in the presence of a vertical gravity vector and causes fish to tilt and move to the

light source. Goldfish aboard the 15-day IML-2 mission exhibited backward "looping" throughout the mission while swordtail fish (*Xiphophorus hefleri*) showed forward looping. On short term parabolic flights gravity changes initiate looping and escape-responses. Ricefish (Medaka, *Oryzius latipes)* also show these behaviors but successfully performed complex mating behavior, and their eggs hatched normal fry in space [Ijiri 2004]. The threshold value for gravity detection by Medaka fish was determined during parabolic flights using a turntable to generate a gradient field of force. The transitions to looping behavior occurred at gravity levels of 0.21 to 0.26 G suggesting these values as thresholds for the fish to sense gravity [Hosoi et al. 2003]. Medaka fish exposed to 3 x **g** significantly increased c-fos expression 30 min after the start of a 3 x **g** exposure suggesting that a stress-like response was elicited. The distribution of c-fos transcripts in fish brains was localized to brainstem regions related to vestibular function [Shimomura-Umemura et al. 2006].

Amphibians and Reptiles. Japanese tree frogs (*Hyla japonica*) flown on the Mir Space Station when free floating arched their backs and extended their limbs as is observed during jumping or "parachuting" on the ground. Floating frogs could not control their movements for locomotion and orientation. Frogs on surfaces bent their necks backward sharply, pressed their abdomens against the substrates and walked backwards in this posture which resembles that during vomiting on the ground and may reflect motion sickness. Adaptation to microgravity was observed in the landing behavior that occurs after jumping. Readaptation of the frogs to the Earth environment took place within a few hours after return. Histological examinations showed changes in some organs such as spine but not brain [Yamashita et al. 1997; Izumi-Kurotani et al. 1997]. During parabolic flights a striped rat snake (*Elaphe quadrivirgata*) and three striped-neck pond turtles (*Mauremys japonica*) were observed. Flight videos showed that the snake responded to the shift from hyper- to hypogravity by assuming a defensive posture and even struck at itself. The turtles actively extended their limbs and hyper-extended their necks in microgravity which is identical to their contact "righting reflex" when placed upside-down in normal gravity [Wassersug et al. 1993].

Rodents: Cellular and molecular effects. Data from Biosatellite and SLS-l show that rodents exhibited changes in CNS areas receiving proprioceptive, vestibular and visual inputs. The data suggest that microgravity indirectly induces changes in brain areas via decreases in afferent input resulting in reduced activity in motor cortex, and increased activity in visual cortex. In general, the decrease in afferent input in the somatosensory cortex results in reactive synaptogenesis and decreased function associated with decreased synthesis of neurotransmitters and neuropeptides. Several flight and ground-based studies have revealed changes in the number of synapses in rats exposed to altered gravity fields representing adjustments of the CNS to the altered sensory input [Vazquez, 1998]. Reactive synaptogenesis is characterized by the sprouting of intact axons to compensate for synaptic sites lost due to degeneration or death of axons accompanied by a rapid increase of microglial cells and astrocytes. This reaction requires the formation of new axons or dendrites, branches and synaptic contacts. It correlates with the expression of genes including src, NCAM, integrins, transcription factors like CREB, trophic factors like BDNF and its receptors, and structural proteins. The role of glial cells is related to modulation of transmitter uptake or extracellular ion composition. In hind limb unloading studies with rats there was a decreased content of γ-aminobutyric acid (GABA) and increased content of glutamate (Glu) in the hippocampus suggesting an imbalance of inhibitory to excitatory activity. Differential expression of 53 synaptic proteins revealed remodeling of presynaptic SNARE complexes

suggesting altered synaptic vesicle recycling [Wang et al. 2015; 2016]. Microgravity exposure also elicits oxidative stress in the CNS distorting various signaling systems involved in homeostatic functions. In a rat hind-limb suspension model (SM) proteomic analysis found alterations in levels of 132 proteins related to signaling cascades including 14-3-3 systems and calmodulin-dependent protein kinase which were upregulated under simulated microgravity. These proteins are associated with circadian regulation, stress responses and synaptic plasticity [Iqbal et al. 2014]. Oxidative stress is responsible for energy imbalances and cellular damage. In rats subjected to hind limb suspension levels of metabolic proteins in the hippocampus underwent differential expression of 42 and 67 mitochondrial metabolic proteins after 21 and 7 days of SM, respectively. Mitochondrial complexes I, III, and IV were all involved but no obvious cell apoptosis was observed after 21 days of SM [Wang et al. 2016]. Expression of choline acetyltransferase (CAT), neurofilaments (NF), calbindin (CB), neuronal nitric oxide synthase (NOS), caspase 3, and cell division marker Ki-67 in mouse spinal cord motor neurons were determined after a 30-day Bion-M1 biosatellite space flight. Under flight conditions motor neuron size increased, the number of neurons containing CAF and NF decreased while the number of CB-positive neurons increased. NOS and caspase 3 expression increased with the appearance of apoptotic bodies but cell division was static. These results indicated a remodeling of the spinal cord neurons [Porseva et al. 2017].

Rodents: Behavior. Rats' motor reactivity to novelty, fine motor coordination during walking on a ladder, and their swimming performance were evaluated following 14 days of hindlimb unloading. The unloading severely impaired motor activity and skilled walking but had no effect on swimming performance [Canu et al. 2007]. Young male Wistar rats treated for 4 weeks with hind limb suspension were tested for tactile sensory behavior in back paws using von-Frey filaments (aesthesiometry). Peripheral nerve density was unaffected and mechanical hypersensitivity developed in all groups suggesting that restraint stress and inactivity were responsible [Tanaka et al. 2013]. Rats monitored during parabolic flights during flight trajectories customized to generate graded levels of partial gravity (between 0.4 and 0.2 x **g)** showed startle and crouching; hindlimb stretching emerged at 0.15 **g** and was more frequently observed at levels approaching 0.01 **g**. It was suggested that different thresholds may exist for emotional and balance/posture-related behaviors [Zeredo et al. 2012]. Behavioral investigations under 2 x **g**, have shown maze performance to be significantly impaired in rats suggesting that animals need a constant gravity reference in spatial learning [Mitani et al. 2004]. In mice elevated spontaneous activity occurred and was correlated with brain levels of nerve growth factor (NGF), and brain-derived neurotrophic factor (BDNF). NGF was affected more than BDNF [Francia et al. 2004]. There are possible complications from rotation when using centrifugation models of hypergravity. Rats were assessed for evidence of rotation sickness by monitoring standard behavior tests, and changes were observed whose magnitude scales with the duration of the rotation for up to 12 hr with some recovery later [Cai et al. 2005]. In rats exposed to both hind limb unloading and irradiation with gamma rays or a broad energy spectrum of protons, prefrontal cortex and hippocampus exhibited changes in monoaminergic and cholinergic neurons accompanied by decreased thigmotaxis and working memory but not spatial memory. Acetylcholine levels in hippocampus were especially responsive to combined treatments [Kokhan et al. 2017]. Gerbils (*Meriones unguiculatus*) moved chaotically throughout flight (Foton) without attempting to stabilize positions by grasping the wire mesh of the cage system (unlike mice and rats) [Il'in et al. 2009].

Birds. When pigeons with various inner ear lesion combinations or with eyes covered were released in weightlessness birds with one obstructed labyrinth showed a barbecue spin rotation. Birds with vertical canal blocks showed rotatory movements in the plane of the blocked canals. In weightlessness they made tumbling movements that resulted in a spiral flight pattern. In birds with both labyrinths obstructed three different flight components could be distinguished, linking specific illusions to specific vestibular end-organ lesions [Oosterveld et al. 1975, 1987].

Non-Human Primates. The performance of two rhesus monkeys (*Macaca mulatta*) in performing an eye-head-hand coordination task was investigated during the 14 d Bion 11 satellite flight. The animals were also trained on the Psychomotor Test System, a package of software tasks and computer hardware developed for spaceflight. They were tested in the task before and after the satellite flight. Flight monkeys showed a significant disruption in performance [Washburn et al. 2000]. And, in the coordination task, comparison to the ground controls, it took the flight monkeys more time to locate and reach light stimuli located 40 degrees to the left or right of a center of the test apparatus panel. During flight days 5-6, the precision of hand movements deteriorated but improved from days 7-14 [Antsiferova et al. 2000].

Humans. The responses of humans to space flight factors has been studied for over 50 years and numerous CNS mediated changes are observed which can impact crew performance, health and safety. Microgravity, ionizing radiation, absence of circadian rhythm, confinement and isolation are but a few of the influences on human behavior and physiology. Central nervous system changes occur during and after spaceflight and are manifested as neurovestibular problems, alterations in cognitive function and sensory perception, and psychological disturbances. These have been extensively reviewed by Cassady et al. [2016] and are consistent with observations with animals discussed above, validating the use of model systems and the importance of understanding the underlying physiology.

Understanding the effects of space radiation on the CNS is important. The combined effect of space radiation exposure with other spaceflight factors on acute and late CNS adverse functional changes and neurodegenerative disease risks is unknown. Other spaceflight stressors contributing to behavior and cognitive risks include isolation, hostile/closed environment, distance from Earth, and altered gravity. These hazards are of concern because they contribute to psychological and physical stress or modified behavior (affect), sleep deficiency, altered circadian rhythm, hypercapnia, chronic inflammation, and altered immune, endocrine, and metabolic function [Strangman et al., 2014]. National Council on Radiation Protection and Measurements (NCRP) report # 153 [NCRP, 2006] and several reviews [Obenaus et al. 2012; Wong et al., 2004; Tofilon et al. 2000; Schultheiss et al.,1995] have summarized known high-dose responses of the CNS to radiation. But, these do not properly predict the effects of space-like low-dose, low-dose-rate exposures to mixed fields of charged particles. Recent reviews of evidence for space-like radiation fields and low-dose photon studies [NCRP 2014; NCRP, 2006; Nelson, 2009, Cucinotta et al. 2014] conclude that there is now convincing evidence for significant alterations in behavioral, neurogenic, neurochemical, inflammatory, and electrophysiological changes to the CNS elicited by space-like radiation fields generated by accelerators. Acute (during missions) and late CNS risks from space radiation are of concern for exploration missions. Acute CNS risks include changes in cognition, motor function, behavior, and mood, which may affect performance and human health. Specific examples of human behaviors and cognitive function of interest that may be affected by space flight include short-term memory, learning, spatial orientation, motor function, emotion recognition, risk decision

making, vigilance, reaction time, processing speed, circadian regulation, fatigue, and neuropsychological changes [Strangman et. al 2014]. The late CNS risks are possible degenerative neurological disorders such as AD, dementia, and premature aging.

Radiation Effects and the CNS. Potential acute and late risks to the CNS from galactic cosmic rays (GCR) and solar particle events (SPEs) are an active concern for NASA [NCRP, 2006, NCRP 2014]. The charged particle component of space radiation represent a unique environment unlike terrestrial forms of radiation. Concern for CNS risks originated with the light flash phenomenon from cosmic ray traversals of the retina which were observed by the Apollo astronauts. GCR are capable of producing a column of heavily irradiated and potentially damaged cells along their path through tissues, raising the concern over serious impacts on the CNS. Many experimental studies using heavy ion beams simulating space radiation provide evidence of the CNS responses to space radiation mostly based on rodent models. Exposure to high atomic number Z, high energy (HZE) nuclei at low doses (10-50 cGy) have now been demonstrated to induce neurocognitive deficits in several mouse and rat behavioral paradigms but equitoxic doses of gamma rays and X-rays do not necessarily show similar effects. Exposure to HZE disrupts neurogenesis in the hippocampus, generates reactive oxygen and nitrogen species (ROS/RNS) in tissue, and increases levels of neuroinflammatory markers with associated activation of microglia. Recent studies show persistent reductions in neuron arborization and synapse number (dendritic spines) from doses 10 cGy and electrophysiological properties of individual neurons and functionally integrated populations of neurons show impairments below 25 cGy of protons and HZE. Finally, studies using transgenic mice developing neurodegenerative pathologies similar to Alzheimer's disease find that low doses of charged particles may accelerate the pathological processes and augment their severity.

Quantification of CNS radiation. Both GCR and SPEs are of concern for CNS risks. In deep space, GCR doses and dose equivalents of more than 0.2 Gy and 0.6 Sv per year, respectively, are expected [Cucinotta 2006; 2014]. The fluence of charged particles hitting the brain has been estimated and suggests that during a 3-year mission to Mars at solar minimum, 20 million out of 43 million hippocampus cells will be directly hit by one or more particles with charge $Z>15$ [Curtis et al., 2000]. Parihar et al. [2015] provide another calculation of traversal frequency for several neuron structures suggest that most dendritic trees will be traversed while individual dendritic spines will not be. This does not include the additional cell hits by energetic electrons (delta-rays) produced along the track of HZE nuclei [Cucinotta et al., 1998]. Norbury et al. [2014] and Slaba et al. [2014] estimated that within a spacecraft with $\geq$10 g/cm^2 shielding, the dominant contributions to dose at all locations in the human body will come from protons and helium nuclei. Further, the average traversals per cell nucleus per year will be $\approx$ 126 and 7 hits per cell nucleus for H and He, respectively vs half of cells for all HZE.

Radiation Effects in Animal CNSs. In animal models irradiated with space-like radiation fields the proliferating population of neurons in the hippocampus is inhibited from reproducing, and patterns of differentiation are altered. This prevents new neurons from integrating into circuits associated with learning and memory. Persistent oxidative stress develops along with inflammatory responses to generate an altered microenvironment for the neuronal network. The blood capillary network undergoes a reversible decrease in its connectivity with likely reductions in tissue oxygenation. Low doses of many different particles can result in the remodeling of neurons such that the complexity of their dendritic branches and the number of their dendritic

spines (and associated synapses) are reduced, which would interfere with information processing. Electrical properties of individual neurons and their cell membranes are altered, and the ability of neurons to transfer information from one to another across synapses or to strengthen their connections after stimulation is impaired. Levels of numerous molecules associated with synapse structure, ion movements across membranes, inflammatory signaling, cell survival, and DNA repair are altered. There is an impairment of the ability of the tissue to recycle damaged proteins. Most importantly, these changes are associated with alterations in behaviors reflecting cognitive abilities and memory. The dose responses can be complex and non-linear. There are regional differences in tissues, and effects are sex-, age-, species-, and genetic background-dependent. Overall, the evidence points to persistent measureable changes in the functional status of the CNS similar to those seen during aging and in some neurological diseases. In the case of neurogenesis, pluripotent neural precursor cells are the most radiosensitive cells of the mammalian brain [Tofilon and Fike, 2000]. Studies with low-LET radiation show that radiation impairs not only proliferation of neural precursor cells but also persistently disrupts their differentiation into neurons [Rola et al., 2004]. Unlike in adults, neural precursor cell death is widespread after irradiation in brains of developing rodents and fish, these studies have been used to estimate RBEs with values from 1.4 to 9.8 for C & Fe ions as well as neutrons [Ishida et al. 2006; Yasuda et al., 2011] Contributions of impaired neurogenesis to overall cognition are not yet well established.

Radiation-Induced Oxidative Stress, Inflammation and Molecular Markers. *In vitro* studies using cultured rodent neural precursor cells from the hippocampus show an increase in reactive oxygen species following X-ray or proton exposures after 6 to 24 hours [Giedzinski et al., 2005]. High-LET radiation led to significantly higher levels of oxidative stress compared to lower LET. Tseng et al. (2014) demonstrated persistent oxidative stress in H-, O-, Ti-, and Fe-irradiated mouse and human neural stem cells at < 1 cGy, Baulsch et al. [2015] extended these observations using cultured human neural stem cells. *In vivo* radiation exposure is associated with acute and chronic elevation of oxidative stress. In mice, persistent oxidative changes are induced by low doses of charged particles; fluences at less than one ion traversal per cell nucleus were sufficient to elicit radiation-induced oxidative stress [Tseng et al., 2014]. Inflammation disturbs CNS function and is mediated by altered activation states of microglia and astrocytes, interruption of the blood brain barrier, and local expression of a wide range of inflammatory mediators, including pro-inflammatory cytokines, chemokine receptors, and adhesion molecules [Tofilon and Fike 2000]. Microglial activation and inflammatory cytokine production have been implicated in cognitive deficits [Jenrow et al. 2013]. Elevated inflammatory markers have been observed in many studies using charged particles. Altered gene expression in brain tissue has been shown to be dose-, dose rate-, and radiation species-dependent and involves neurotrophins, receptor ion channels, and genes regulating synaptic plasticity, vascular function, oxidative stress and amyloid processing as well as microRNAs [Chang et al. 2010; Khan *et al.* 2013; Lowe et al., 2009; Kempf et al. 2014]. Proteomic analysis of irradiated mouse and rat brains showed changes in many peptides [Lim et al. 2011; Britten, 2010]. Changes were observed to persist for > 6 months. Regulation of protein homeostasis via proteasome and autophagosome activity have also been shown to be impaired by charged particle exposure [Poulose et al. 2011] and were associated with glial cell activation, oxidative stress, and inflammation for up to 75 days.

Microvascular and Neuronal Structural Changes and Electrophysiology. The topology of neuronal networks and structural plasticity are important regulators of cognitive performance, as they control synapse number, strength, and organization. Recent neuronal morphometry investigations in neurons have demonstrated that radiation causes reductions in hippocampal neuron arborization as well as loss of dendritic spines at ≥ 10 cGy, each of which would limit the complexity of signal processing [Chakraborti et al. 2012; Parihar et al., 2015b]. Late necrotic brain tissue damage after high radiation doses is associated with damage to the vascular system [Tofilon and Fike 2000] and new evidence suggests that low doses of charged particles can disrupt vascular structure and function. Mao et al. [2010] demonstrated substantial microvessel loss at 9-12 months in the mouse hippocampus after 0.5 - 2 Gy of H or Fe exposure. Microvessel disruption can be associated with blood brain barrier breakdown and poor tissue perfusion. Electrophysiological experiments with low doses of charged particles have revealed that both intrinsic properties and synaptic parameters change. In rodent acute brain slices (usually the hippocampal field) extracellular field recordings show that synaptic transmission is altered by H, O, Si, and Fe ion exposures with complex dose and ion species dependence. Excitability, presynaptic glutamate release, recurrent inhibition, synaptic efficacy, long-term potentiation (a tissue-level model of memory formation), and other measures exhibit neuronal field-, dose-, and ion-specific modulation consistent with dysregulation of the balance between excitatory and inhibitory activities post-irradiation [Vlkolinský et al. 2008, 2007]. Single cell patch clamp studies revealed that proton exposures hyperpolarized cell resting membrane potentials, decreased input resistance, and upregulated persistent sodium currents which together lead to a reduction in neuron excitability [Sokolova, et al. 2015]. There is evidence that inhibitory neurons may be more sensitive to radiation than excitatory neurons and it has been demonstrated that different inhibitory neuron subclasses exhibit unique responses with respect to connectivity and excitability [Lee et al., 2016].

Effects of Radiation on Behavior and Neurodegeneration. The most commonly employed rodent behavior tests have included the Morris water maze and Barnes maze [Britten et al. 2012; Villasana et al. 2010], novel object recognition, object in place recognition, [Kumar et al. 2013; Shukitt-Hale et al. 2000; Tseng et al. 2013], and contextual fear conditioning [Raber 2013, 2011] for hippocampus-dependent memory but with strong associations with the cortex as well. Cognitive behaviors more closely associated with the frontal cortex include operant conditioning [Rabin et al. 2007; Rice et al. 2009], attentional set shifting [Britten et al. 2014; Lonart et al. 2012], and psychomotor vigilance tests [Heinz et al. 2008; Davis et al. 2014]. Anxiety and fear are commonly assessed with open field tests and elevated plus or zero mazes [Kumar et al. 2013]. Many other tests have been employed as well, such as acoustic startle [Haerich et al. 2005]. Radiation types investigated include X-rays, gamma rays, electrons, and accelerated ions (H, C, O, Si, Ti, and Fe, with energies from 150 MeV/n to 5 GeV/n. Detection limits for some tests approach 1 cGy. When transgenic mice overexpressing human Alzheimer amyloid precursor protein were exposed to low doses of accelerated iron ions, the radiation accelerated the appearance of age-related electrophysiological properties, decreased cognitive abilities (contextual fear conditioning and novel object recognition) and accelerated of Aβ plaque pathology [Vlkolinsky et al. 2010; Cherry et al. 2012].

Radiation Effects in the Human CNS. Deleterious effects of ionizing radiation on the human CNS have been observed in radiotherapy patients receiving high localized doses [Greene-

Schlosser, 2012a,b] far above doses to be encountered by space travelers. Neurocognitive effects are observed at lower doses, especially in children [Schultheiss et al., 1995]. In lower dose whole-body exposures for treatment of childhood leukemia, adult survivors exhibit deficits in information-processing speed, memory, attention, and learning [Armstrong et al. 2013]. Atomic bomb and Chernobyl accident victims receiving low to moderate doses of radiation ($\leq$ 2 Gy) also show evidence of memory and cognitive impairments, more frequent psychiatric disorders and altered electroencephalographic (EEG) patterns [Yamada et al., 2009; Loganovsky, et al., 2001]. A-bomb survivors [Yamada et al. 2009] did not exhibit increased risk of radiation-associated dementia, but mental retardation was observed in children exposed prenatally during the early post-conception period [Otake, 1998].

SPECIFIC SCIENCE

There is a pressing need to understand the underlying mechanisms in order to mitigate deleterious effects to humans and other organisms that may accompany them on deep space missions. Such investigations will also inform our knowledge of how animals' anatomy and physiology are organized and regulated in a constant 1 x **g** environment and across the gravity continuum.

1. How does elimination of the gravity vector as a stationary reference alter sensory input, processing and resultant motor responses?
2. How are sensory inputs affected by convection, diffusion and proprioception which may confound strengths, orientations and gradients of chemical and mechanical signals?
3. When gravity levels drop from 1 x **g** to 0 x **g** the vestibular system dominates adaptation but upon return to 1 x g the visual system seems to dominate. Will this be true for Moon and Mars level gravities? Which neural circuits control these adaptations, what is their sensitivity and what are the time courses of the adaptations?
4. Are there thresholds for gravity detection or is it a continuum? Why do some behaviors change discontinuously as gravity levels change?
5. There are changes to neurogenesis in the hippocampus and olfactory bulb of mice under microgravity. Is this due to changes in the physiology of the neurogenic micro-environment or a reaction to activity and environmental enrichments? Or is it a stress response? What are the impacts on memory and cognitive functions?
6. How do hippocampal (mushroom body) neurons in *Drosophila melanogaster* respond/change with microgravity and/or radiation? What are the underlying genetic determinants that regulate these responses?
7. Specific neurons have been shown to exhibit altered gravity-induced functional changes in nematodes and crickets. These are motor neuron axons (worms) and a gravi-sensing neuron (cricket). How are these plastic changes elicited and what inputs to the dedicated neurons drive the responses? Are such dedicated structures present in other systems?
8. In the statocyst model of gravisensing, a mass is thought to interact with mechanosensitive membrane ion channels to transduce responses of the cell. The mass may be a mineralized structure or the cytoplasm itself. How valid is this model and what are the biochemical and structural components?

9. Sensory deprivation during critical developmental periods may lead to reversible or irreversible sensory processing. Does reduced gravity and its indirect effects on the physical environment (convection, etc.) result in sensory deprivation across the gravity continuum? Does this occur in mature as well as developing organisms?
10. Do spaceflight hardware environments for humans and animals impose unavoidable constraints on normal CNS function leading to maladaptation (e.g. lighting, noise, reduced volume for movement)? What parameters are the most significant?
11. Vestibular system function is reflected in body orientation and posture. How do the resulting body configurations and stereotyped reactions to gravity vector arise and how do these programs react to partial gravity levels?
12. Is there structural adaptation to weightlessness in terms of reactive synaptogenesis or plasticity? What are the synaptic, glial or extracellular components involved and how do they relate to immune system components? Do microglia actively participate?
13. Is gravity a continuum for neural processing? Are there thresholds of hypogravity? How do hypergravity fields modify responses? Do reactions to gravity levels scale linearly, non-linearly or discretely?
14. What are the interactions of the CNS with other systems, especially the immune system in its adaptation and compensation to altered gravity levels?
15. Behavior results in activity dependent plasticity. Are "hard-wired" or stereotyped plasticity responses used in novel environments or are new programs developed. What are the limits of the adaptation.
16. What is the role of epigenetics in adaptation to microgravity? Do methylation, histone modification, and microRNAs play significant roles in this adaptation. Is it transgenerational like diet and stress can be? Are there multigenerational changes?
17. What changes in gene expression occur and how do they manifest themselves in neural information processing?
18. What changes occur to neurotrophins, metabotrophic ion channels and transcription factors in space flight that control adaptation?
19. Flies are thought to encode gravity, sound and air motion by related mechanisms. Are these generic mechanical sensing mechanisms?
20. How do motor programs adjust to altered body strength (musculoskeletal wasting) and diminished restoring forces?
21. Geotaxis and gravitaxis interact with phototaxis. How are mismatched inputs resolved?
22. Are there sex differences in CNS responses to spaceflight factors?
23. Do nervous systems self-calibrate against a dynamic environment? For example, mismatched otolith sizes are compensated by the brain. How is this accomplished, what is the time scale and is it reversible?
24. Social stresses and group interactions can affect behavior. How do spaceflight environments interface with social interactions? Can adaptations to the spaceflight environment be taught?
25. How are integrated sensory cues interpreted and resolved when body orientation is unrestricted but visual cues are fixed?
26. How well do microgravity simulations like hind-limb unloading predict actual responses to weightlessness as the underlying physiology is not the same?
27. How important are alterations in thigmotaxis, such as being able to grip substrates when contact forces are reduced?

28. Flight, swimming, and other locomotion disturbances are commonly observed and compensation strategies develop in most but not all systems. What limits plasticity in these situations?
29. Do activity dependent plasticity mechanism such as long term potentiation and depression proceed normally in space flight environments?
30. Do habituation and extinction occur normally?
31. Can cognitive or other behavioral tests be designed to work in space so as to validate ground-based predictions?
32. How do altered circadian cues, sleep disturbances, elevated pCO_2 etc. interact with microgravity and radiation-induced CNS changes?
33. Are effects observed after acute radiation exposures to single ions also seen after protracted exposures and exposures to mixtures of charged particles?
34. Are their common biochemical and biological pathways shared by exposure to radiation and other spaceflight stressors? Are oxidative stress and inflammation such pathways and what are their relative contributions to outcome measures? Would combined exposures be expected to be additive or synergistic?
35. Is radiation-induced damage to CNS repaired normally in microgravity? What are the important targets in neural tissue: cell nuclei, soma, highly branched long processes, or extracellular matrix?
36. Do fluid imbalances adversely affect the "glymphatic system" which regulates production, transport and clearance of cerebrospinal fluid?
37. Are blood brain barrier, blood retina barrier and blood spinal cord barriers intact in space and do they maintain immune privileged compartments?
38. Which are the most radiosensitive cells? Neural stem cells, excitatory neurons, inhibitory neurons, glia, endothelium, innate immune system? What are their dose, ion type and dose rate dependencies?
39. What are the influences of non-CNS organs and tissues on CNS function in space flight? Fluid balance, perfusion, endocrine and metabolic states all are likely to interact in a bi-directional fashion with the CNS.
40. Do altered microbiomes in space affect CNS function?
41. Is the architecture of the brain, particularly the gravity sensing system, shaped by gravity?
42. Similarly, is the development and architecture of motor and sensory systems dependent upon gravitational input?

CRITICAL TECHNOLOGIES

Critical technologies are those that enable the production and testing of animals in variable gravity levels and produce space-like radiation environments either together or in combination. The Brookhaven National Laboratory and hadron radiotherapy facilities are critical for simulating radiation environments and are currently working to produce mixed ion environments delivered at low dose rates or in multiple fractions. Access to hypergravity facilities such as the NASA ARC and university-based centrifuge facilities are critical as are validated reduced gravity models such as the hind limb unloading model, clinorotation and random positioning systems. Methods to combine environmental stressors in a protracted setting are highly desirable. Frequent access to space flight and, when applicable, short term parabolic flight environments are also desirable. For assessing psychological effects of confinement,

isolation, circadian disruptions, etc., in humans, analog environments also have a key role to play but animal-specific homologs are not currently well-defined or readily available.

GOAL 7— AN INTEGRATED APPROACH TO UNDERSTAND THE IMPACT OF DEEP SPACE MISSIONS ON DEVELOPMENTAL BIOLOGY

This goal seeks to address the molecular, structural and physiological processes that control the growth, differentiation, adaptation and reproduction of organisms in the context of the physical environment in space and adaptations elicited by transitions between environments. In the context of the space environment, alterations in gravity levels, radiation exposure and artificial situations imposed by life support systems are the dominant factors that define the limits and constraints on the physical environment. Development and reproduction are two of the oldest areas of investigation in biology and cover all periods of lifecycles and the transmission of information across generations of organisms of all types. This knowledge applies to adult stem cells and cell biology. Technological advancements have steadily driven investigations towards molecular and genetic levels of detail while maintaining attention to physiological and system level controls and responses. Model systems with short life spans, complex organization and behavior, and for which sophisticated genetic manipulations are available are particularly attractive for developmental studies in practical terms and complement vertebrate studies for which limited phases of life cycles have so far been addressed.

BACKGROUND

A free return trajectory from Mars would exceed the developmental time for a human fetus. For young and developing organisms raised in microgravity, altered sensory inputs or sensory deprivation may lead to structural differences that impair adaptation to normal 1-**g** ambient conditions. Early studies of development and reproduction already focused on the issue of whether the Earth's omnipresent gravitational field imposed limits on the form and development of organisms. Investigations related to how mechanical forces determined body axes employed centrifugation or reorientation of large embryos from invertebrates and amphibians as far back as the late 1800's. The scaling relationships in terms of body size, mechanical structure and strength, and energy balance have also been subjects of investigation for well over a century. Modern investigations have focused on control systems driven by genetic programming, complex signal transduction mechanisms, the molecular specification of spatial information, concepts related to plasticity and adaptation and more refined manipulation of the physical environment. Only in the space age did it become feasible to manipulate gravity levels below 1 x **g** and was it recognized that the space environment also had a significant radiation component. Experience in spaceflight also lead to the recognition that the effects of other physical forces manifested in different ways in the absence of gravity, e.g. convective mixing. The basic issues recognized early on continue to be emphasized in modern research program plans and many comprehensive reviews of flight and ground based experiments are available [Alwood et al., 2017; Marthy 2009; NRC, 2011].

Reproduction. Space flight experiments have established that complex organisms can live and grow under weightless conditions (in the presence of modest radiation exposures). Some

invertebrates and microorganisms have completed multiple generations in space [Mashinsky, 1994; Oczypok et al., 2012] while vertebrates and mammals have completed all segments of life cycles [Horn and Gabriel, 2014; Murata et al. 2015] but certain special circumstances arise. For example, maternal - offspring behavioral interactions related to the artificial environments in space may limit successful postpartum development in mammals [Ronca et al. 2008]. Similarly, modifications to mass transport processes (e.g. convection and diffusion) secondary to low gravity may influence aquatic organism development indirectly [Warren et al. 2013] and cell-scale physical forces such as surface tension may overwhelm the influence of much weaker gravity [Albrecht Buehler, 1991]. The structural and biochemical features of cells and tissues are robust, dynamic and under multiple levels of control that confer resistance to perturbation by gravitational forces. For example, functions of highly specialized gametes are sufficient for successful fertilization [Tash and Bracho, 1999]. Ricefish (Medaka, *Oryzius latipes)* successfully performed complex mating behavior, and their eggs laid in space developed normally and hatched as fry in space [Ijiri, 2004].

Development. Altered developmental patterns have been observed in organisms "exposed" to weightlessness for various periods of time at different stages of their life cycles. The deviations in the stereotyped sequence/stages of developmental events manifest as altered process timing, gene expression patterns, temporary anatomical differences that may later resolve, but sometimes result in permanent changes such as reduced body sizes of normal architecture [Leandro LJ et al., 2007; Ma et al, 2015; Xu et al. 2014]. The underlying causes may include different balances between stem cell kinetics, cell growth, and programmed cell death or senescence [Blaber et al., 2014]. Behavioral changes in animals suggest the existence of critical periods in which sensory inputs (e.g. vestibular system) are required for normal establishment of neural pathways [Anken, 2003; Krasnov, 1994] and for which sensory deprivation leads to "impaired" performance which might also represent valid behavioral programs appropriate to the weightless condition but not to 1 x g [Ibsch, et al. 2000]**.** Appropriate integration of multiple sensory inputs compounds this issue and a human-relevant example is space motion sickness. Meanwhile, some gravity detection mechanisms have been described in microorganisms, plants and animals that regulate growth patterns (e.g. shoots up and roots down in plants) and behaviors (e.g. gravitropisms) while others remain poorly characterized [Kiss et al. 1998; Hemmersbach et al, 1996]. Evidence suggests that observable gravity-dependent effects scale continuously with force levels. This lends relevance to tractable hypergravity investigations to complement studies of hypogravity responses which are more technically limited [Wade, 2005]. It is not yet established whether low gravity response thresholds exist.

Developing Rodent Nervous System. In embryonic rats that developed in microgravity, while axons of vestibular sensory neurons reach their targets in microgravity, development of terminal branches and synapses is delayed reflecting the environmentally controlled phase [Ronca et al. 2000] suggesting that the vestibular sensory system has genetically programmed processes of development that establish general patterns of connectivity between the CNS and the periphery that environmental stimuli regulate during activity to fine tune synapses in microcircuits. The developing CNS is robust in adapting and compensating as evidenced by the observation that newborn rat pups were able to suckle dams during short parabolic flights and on the Neurolab mission, where pups showed milk-letdown reflexes, stretching and hindlimb extension while remaining attached during periods of microgravity and hypergravity [Ronca et al. 2013]. The postnatal development of sensory systems has been shown in studies over the last 40 years to be

influenced by experience during critical periods of development. For example, swimming behavior of young rats reared from postnatal days 14 to 30 in microgravity was altered [Walton et al. 2005]. The data suggest that the most fundamental of these adaptations is a resetting of the basic motor rhythm to a higher frequency.

Development and gene expression. Multigenerational growth of *Drosophila melanogaster* in space have shown that while fertility is maintained, there are several changes in cytoskeletal proteins and altered transcription of genes involved in morphogenesis, cell differentiation, metabolism and proteolysis [Ogneva et al, 2016]. Other studies involving proteomics and transcriptomics under altered gravity conditions also point to perturbation of several important physiological systems within developmentally important life stages of *Drosophila* [Hateley et al. 2016, Hosamani et al. 2016]. *D. melanogaster* experiments on the space shuttle showed an increase in the frequency of lethal mutations induced by spaceflight as measured by a sex-linked recessive F2 screen on the male germline [Ikenaga et al. 1997]. Other studies used the stick insect, *Carausius morosus*, to show that the combined effect of microgravity and radiation had the greatest effect in inducing developmental body anomalies in spaceflight larvae compared to either spaceflight 1-g controls or ground controls [Reitz et al, 1989] . Invertebrate models will therefore provide valuable data for deep space environments, where radiation and microgravity may both be important factors in inducing developmental changes on biological systems.

Epigenetic adaptation. Observations in microgravity focus attention on the continuous influence of the environment associated with space flight conditions. Organisms must constantly adapt to and compensate for environmental conditions. Their behavior, energy requirements, posture, orientation, interactions with solid - liquid - gas interfaces, communication, sensory inputs, management of food and waste balance, etc. all impose burdens on normal homeostasis. The state of organisms in space may be normal but reflective of alternate set points or balanced compensatory and adaptive processes within the repertoire of the organism [Alberts & Ronca, 2005]. Recent work has drawn attention to the fact that epigenetic mechanisms may help to stabilize homeostasis and adaptive processes and that transgenerational epigenetic mechanisms may be able to transfer adaptive advantages to offspring based on the environmental experiences of the parents [Boyko & Kovalchuk, 2011; Jablonka, 2009]. An exceptional example of this is an observation in *C. elegans* that epigenetic tags associated with certain environmental stress responses may persist for up to 14 generations [Klosin et al, 2017].

Circadian Rhythms. Altered gravity load induced by spaceflight and centrifugation (hypergravity) is associated with changes in circadian, metabolic, and reproductive systems. For example, exposure of rats to 2 x **g** hypergravity during pregnancy significantly changed expression of core clock genes in mammary and liver tissue and circadian rhythms of maternal behavior [Casey et al., 2012]. Invertebrates such as *Drosophila melanogaster* can play an important role in elucidating the genetics and signal transduction changes induced by the BLEO environment on circadian rhythms as this model system has played an important role in elucidating circadian rhythms in all animals (2017 Nobel Prize).

Radiation and Development. A-bomb survivors [Yamada et al., 2009] did not exhibit increased risk of radiation-associated dementia, but mental retardation was observed in children of the atomic bomb survivors in Japan if exposed prenatally during the early post-conception period [Otake, 1998]. *Zea mays* exposed to space radiations or heavy ions from accelerators developed

streaked or split cotyledons upon subsequent germination suggesting the elimination of at least one cotyledon cell in the dormant embryo.

Development and reproduction encompass all aspects of biology from molecular control of metabolism to cell growth and differentiation, to cell and tissue structure specification, the processes of aging, and organism behavior in response to environmental inputs. The responses of these processes to altered gravity levels and artificial environments is fundamental to understanding short term homeostatic regulation and long term evolution of organisms successfully adapted to life in Earth's environment. To predict the successful adaptation of life to environments beyond LEO it will be necessary to appreciate the dynamic reactions to spaceflight environmental parameters at all levels of biological organization including reproduction and development.

SPECIFIC SCIENCE

1. Is there a gravity continuum for biological responses or are there thresholds and discrete transitions?
2. Determine the dependence of cell growth and differentiation on the level and duration of gravity exposure.
3. Determine how altered gravity affects metabolism.
    a. Energy requirements and scaling with restoring forces.
    b. Altered catabolic/anabolic ratios. Kinase/phosphatase balance.
    c. Thermal regulation. Homeotherms versus poikilotherms.
4. Determine whether gravity influences the mechanisms for establishing spatial information in cells and tissues.
    a. Are body axes properly specified?
    b. Cell polarity in embryos. Maternal effects or parental structural templates.
    c. Tissue polarity in limb buds
    d. Does gravity orient subcellular structures? Cytoskeleton, membranous organelles, spindle/centriole, MTOC.
    e. Chemical gradients of signaling molecules.
    f. Role of homeobox genes and associated effector mechanisms.
5. Determine the degree to which altered mechanical loading leads to altered growth, musculoskeletal system configuration, mechanical strength, and motor programs: Cytoskeletal redistribution, tensegrity, compression vs tension forces.
6. To what degree does altered mass transport or reordering of the relative importance of physical forces (convection, diffusion, surface tension, etc.) in different gravity environments affect growth and differentiation?
    a. Concentration gradients.
    b. Nutrient uptake, waste management, chemical communication between cells or organisms.
    c. Heat transfer and energy requirements.
7. Does the suite of adaptive responses to altered gravity represent a stress response? Are there multiple independent responses or an integrated response?
    a. Reactive oxygen and nitrogen species.
    b. Inflammation.
    c. Protein and organelle homeostasis.

d. Mitochondria, lysosomes, autophagy, proteasomes, chaperones, unfolded protein response.
e. Interactions with radiation exposure.
f. Interactions with behavioral stress. Confinement, circadian decoupling, isolation.

Do accumulation of stress effects lead to epigenetic modifications? Do stereotyped 1g behaviors lead to inappropriate outcomes in altered gravity that evoke stress responses?

8. How do altered gravity levels and transitions affect gene expression and regulation?
    a. Gravity level dependent signatures.
    b. Stress response signatures.
    c. Developmental stage specificity.
    d. Sex and age dependence.
    e. Regulation by splicing, specialized RNAs, transcription factors.
9. Are there epigenetic changes to the genome in responses to altered spaceflight environments that may improve or stabilize long term adaptation of an organism, or confer survival advantages to subsequent generations?
    a. Methylation, histone modifications, specialized small RNAs.
    b. Transgenerational transmission. Influence of genomic damage burden from radiation.
    c. Are these reversible?
10. How are signal transduction mechanisms influenced by altered gravity?
    a. Transcription control linked to focal adhesion junctions.
    b. Neurotrophins, growth factors and hormones, cytokines, chemokines, ligand transport.
    c. Altered extracellular matrix composition. Water content dependence on gross fluid redistribution.
11. Do altered gravity levels lead to differences in apoptosis, proliferation or senescence and the balance between the processes?
12. How do alterations in gravity affect stem cells and stem cell niches (microenvironments)?
    a. Hematopoiesis, neurogenesis, intestinal crypts, hair follicles.
    b. Cancer stem cells.
13. How do altered gravity levels affect wound healing and regeneration?
14. Are there changes in aging and lifespan under altered gravity?
    a. Telomere stability, senescence pathways.
    b. Are scaling relations altered? (human vs mouse-year equivalents)
15. Are there age differences in adaptation to altered gravity levels?
16. Are there sex differences in adaptation to altered gravity levels?
17. How do altered gravity and biological timing interact?
    a. Cell cycle mechanisms and timing during development.
    b. Circadian rhythms and cues.
    c. Estrous cycle.
18. Do mineralization processes function properly in altered gravity?
    a. Hydroxyapatite, barite, silica, epitaxial growth,
    b. Skeletal components, vestibular components.
19. Determine whether there are critical periods in development during which gravity is required for establishing intact and appropriate neural circuitry.

    a. Motor programs.
    b. Spatial awareness.
    c. Hippocampal “place” cells.
20. Is the presence of gravity required to establish and maintain normal processing of sensory inputs?
    a. Altered proprioception due to diminished tactile cue strength or vestibular inputs.
    b. Inappropriate integration of sensory inputs with “unexpected values”.
    c. Impairments in plasticity in interpreting inputs.
    d. Sensory deprivation/altered balance within critical periods may lead to permanent adaptations or deficits.
    e. What are the effects of transitions between gravity levels?
21. What are the effects of altered gravity on animal behavior required for successful reproduction?
    a. Courtship, mating & maternal care.
    b. Dependence on engineered living space.
22. How is the microbiome affected by altered gravity levels? What are the effects on host organisms from altered microbiomes?
23. How do consequences of high LET radiation exposure interact with microgravity exposure? Survival, mutation, oxidative stress, inflammation, DNA repair, apoptosis, cancer.
24. Are there consequences of closed environments that affect development and reproduction?
    a. Hypercapnia, cyclic pCO_2 levels, high humidity.
    b. Accumulation of volatile organics.
    c. Habituation to odors. Behavioral consequences.
    d. Stable aerosols with bioactive components. Microbiome interchanges.
    e. Artificial lighting.

GOAL 8— AN INTEGRATED APPROACH TO UNDERSTAND THE IMPACT OF RADIATION EXPOSURES DURING DEEP SPACE MISSIONS

One of the major concerns, especially for long-term exploration missions beyond the Earth’s magnetosphere, is how to protect astronauts from radiation risks. These risks arise primarily from solar energetic particles (SEPs) and galactic cosmic rays (GCRs). Cosmic rays (GCRs and SEPs) consists of approximately 85-90% protons, and 10-13% helium ions (alpha particles), with the remaining 1%–2% consisting of high atomic number and energy (HZE) nuclei particles and 1% electrons. The particle fluence and intensity are highly dependent on solar activities. GCRs consist of high-energy particles ranging from 10MeV/nucleon to 10 GeV/nucleon and beyond, fluxes of which are modulated by the heliosphere and negatively correlated with solar activity. Solar Particle Events (SPE) are sporadic and difficult to predict, lasting for hours to days, with a significant proportion of relatively lower energy protons and some helium ions. These SEPs are mostly below 150 MeV/nucleon, easily shielded with shielding material or the Martian atmosphere. Figure 1 displays the energy range of cosmic ray particles from various sources.

BACKGROUND

Life on Earth is well protected from these cosmic rays for two reasons: a global magnetic field to deflect energetic charged particles, and the atmosphere. While astronauts on the International Space Station (ISS) in Low Earth Orbit (LEO) are exposed to trapped radiation and GCRs with reduced dose and energy, life beyond LEO (LBLEO) is exposed to mostly much less shielded GCRs/SEPs, and secondary particles generated by the shielding materials, atmosphere, as well as regolith if near the Lunar or Mars surface.

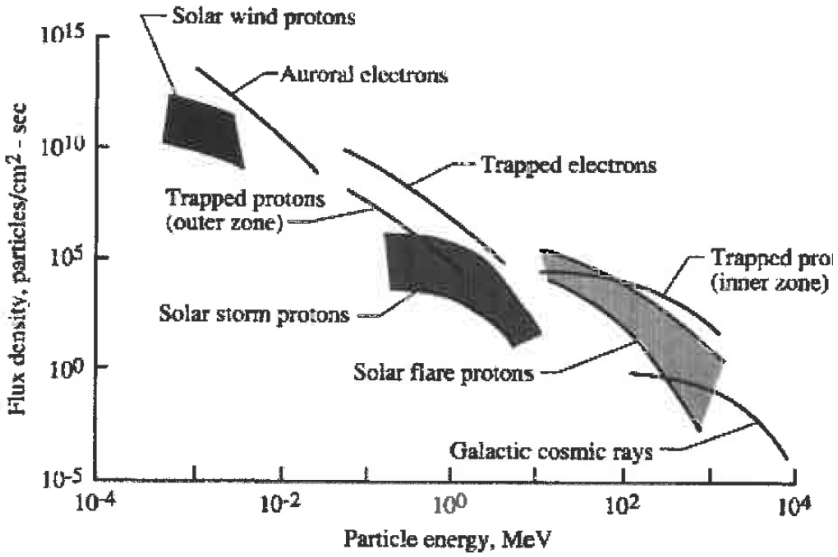

Figure 1. Schematic of energy ranges of space radiation environments [Wilson et al, 1991].

Dose, Dose Rate, and Dose Equivalent. Even though the flux levels of GCR particles are very low, these high-linear energy transfer (LET) particles produce intense ionization as they pass through matter. With less coronal mass ejections and the corresponding earth magnetic field changes during the solar minimum, GCR particles, especially those with lower energy range (<1 GeV/nucleon), have easier access to interplanetary areas and to the surface of the Earth, Mars, and the Moon. Risk estimates are highly uncertain for GCR based on knowledge learned on Earth (NCRP Reports 132). The mean quality factor of the GCR particles is estimated at 3.07 or higher. The quality factors for SPE protons (observed both on the surface and during transit) are Q = ~1-1.5 [Cucinotta et al, 2012]. The quality factor is calculated ultimately based on radiation induced cancer and non-cancer effects in human samples, human cells, and rodents. No conclusive data is available so far for other species (plants, invertebrates, most vertebrates, and microorganisms). The GCR Measurements of dose equivalent rate at different locations are listed in Table 8.1, from the Stratosphere above the Antarctica to the cruise to Mars and on Mars surface.

Table 8.1. GCR Dose Equivalent Rate (mSv/day) at different locations.

Condition	Mars Mission (Mars Science Lab)[1]	Lunar Mission (Apollo)[2]	On ISS (~400 km)[3*]	Sub-orbital (~120 km)[4*]	Stratosphere above Antarctica (30-37 km)[5*]
Transit Journey	1.84±0.33	0.7-3	~0.5 (50-80% from GCR particles)	~0.0035 mSv per 15 min ~0.00031 mSv/15 min (Mercury 3)	0.4-0.6
Surface	0.64±0.12 (Solar min)	0.24-0.30 (Solar max); 0.67-1.04 (Solar min)			

[1] Zeitlin et al, 2013; Hassler et al, 2014; Köhler et al, 2015; [2] Reitz et al, 2012; Durante, 2012;[3] Cucinotta et al, 2012; [4] For sub-orbital flight, the measurement/estimate is total dose equivalent per 15 min flight [Jurist, 2005; Copeland, 2013; Benton, 2012; Möller, 2013] *Total dose equivalent rate, including GCR particles.

These measurements were made at different phases of different solar cycles, which may cause variance based on solar activity intensity. Whole body doses of 1-2 mSv/day and approximately half this value are estimated to accumulate in interplanetary space and on planetary surfaces, respectively [Huff et al, 2016; Cucinotta et al. 2006; Zeitlin et al, 2013]. However, on planetary surfaces there may be an additional contribution from albedo neutrons. The cruise to Mars and on Mars surface was measured near the maximum of solar cycle 24, considered weak by historical norms. The shielding of the lower hemisphere on Mars reduces the dose rate by a factor of ~2. The average quality factor on the Martian surface is 3.05±0.3, compared with 3.82±0.3 measured during transit primarily due to the shielding variance. The effective atmospheric shielding at about 21 g/cm^2 is much thicker than the spacecraft shielding of the Mars Science Laboratory's Curiosity Rover during cruise [Zeitlin et al. 2013; Hassler et al. 2014]. For Lunar missions, there is no atmospheric shielding effect. There is a total estimated mission dose equivalent of ~1.01 Sv for a round trip Mars surface mission consisting of 180 days (each way) and 500 days on the Martian surface for this particular solar cycle [Hassler etl, 2014]. However, the total mission dose of a future deep space mission largely depends on the solar activity by which the GCR flux and SEPs are modulated. Therefore the timing of missions beyond LEO may be considered as a means of dose mitigation.

Shielding Effects and Secondary Particles. While shielding significantly reduces the flux of GCR and SEPs, secondary particles, including neutrons and gamma rays, are inevitable. Because of ineffective shielding and secondary particles, GCR have a significant biological impact, and large biological uncertainties limit the ability to evaluate risks accurately. In general, low atomic-mass materials are preferred shielding against GCR owing to low production of secondary particles [Slaba et al, 2013; Huff et al, 2016]. Unlike GCR, low to medium energy SPE protons can be effectively shielded. However, accurate event alerts and real-time dosimetry are challenging, but essential for crew safety. Secondary particles also include those produced by

Mars or Lunar regolith contributions. A crew member exposed to a significant SPE event with only protection from an EVA suit (0.3 gm/cm^2) has minimal protective shielding; however, this scenario is highly unlikely if operational protocols are successfully implemented where crew would shelter for the majority of event duration, which can last for a few days. The estimated skin dose is considered equivalent to the dose potentially received by plants or other species behind transit vehicle or habitat shielding. Technology exists for mapping doses inside spacecraft and habitats using 3-D material maps, and the application of transport codes to structural designs to be used beyond LEO will be important. An example of such an exercise produced the simulated dosimetry data behind shielding beyond LEO shown in Table 8.2.

Table 8.2. Simulated dosimetry quantities in interplanetary space from total event spectra of GCR, and 1972 large SPE [Cucinotta et al, 2012]. *H* = dose x RBE (or Q).

Dosimetry Quantities	August 1972 SPE (63 hrs)		Annual GCR at Solar Minimum	
	Spacecraft 5g/cm^2	20 g/cm^2 shielding	Spacecraft 5g/cm^2	20 g/cm^2 shielding
H, mSv – Avg Skin	4259	144	832	599

Physical Dosimetry. High Charge and Energy (HZE) particles have unique track structures leading to quantitative and qualitative differences in biological effects compared to γ-rays. To accurately assess the radiation risk, dosimeters with capabilities to detect a wide range of particles and energies should be selected. While absorbed dose is measured via the interaction of ionizing radiation with a detection material and quantified by the energy deposition and transfer to the material, its biological consequences must be measured separately by appropriate biological and biochemical methods. Utilizing several types of dosimeters with different capabilities simultaneously is recommended for radiation measurement during LBLEO missions in order to obtain accurate assessment of the space radiation environment. For example, the light-weight, efficient Radiation Assessment Detector (RAD) has been used extensively in Mars missions and on ISS, which provides valuable real-time measurement. The detector uses a stack of silicon detectors and a crystal of cesium iodide to measure galactic cosmic rays and solar particles and also to identify particles, such as protons, energetic ions of various elements, neutrons, and gamma rays, including secondary particles within a certain energy range. It analyzes pulses to identify each high-energy particle and determine its energy. The RAD used for the Mars missions detects proton flux within the energy range 20 MeV $< E \leq$ 100 MeV, the flux of charged particles with 30 to 200 MeV/nucleon, and Neutrons in the energy range 0.5 to 80 MeV (https://mars.nasa.gov/msl/mission/instruments/radiationdetectors/rad/). A complementary dosimeter for higher energy ranges of particles may be necessary to generate a more precise risk assessment and the simulation of the space radiation environment. In addition to RAD, other passive radiation dosimeters are also useful, such as thermoluminescence detectors (TLD) which are capable of measuring total absorbed dose, and solid state nuclear track detectors (SSNTDs) which are capable of measuring the LET spectrum, fluence and

absorbed dose from charged particles. TLDs provide limited LET information, and their sensitivity to neutrons is dependent on their isotopic composition (e.g. TLD 100 vs. 700), whereas various types of SSNTD made of organic polymers, such as CR-39 or doped sapphire, are the most sensitive models and have been extensively utilized for radiation measurement on ISS.

Track Structure Measurement. Microdosimetry, or measurement of track structure, is also useful for radiation risk assessment. For HZE particles, the occurrence of ionizations and excitations in cells and tissue are not distributed randomly and homogeneously across whole cells and tissues. Therefore, in a complex radiation environment, different types of radiation may deposit in different amounts of energy at the same location in the cell or tissue. The pattern of distribution depends on the type of radiation involved (Figure 2). The energy deposited to the cells or tissue are stochastically produced but localized along the track of the incoming radiation. For instance, the induced double strand breaks (DSBs) in TK6 cells *in vitro* appeared as dense patterns of phosphorylated histone γ-H2AX reflecting tracks of ionizations and excitations along the particle path [Yatagai et al, 2011; Moreno-Villanueva et al, 2017].

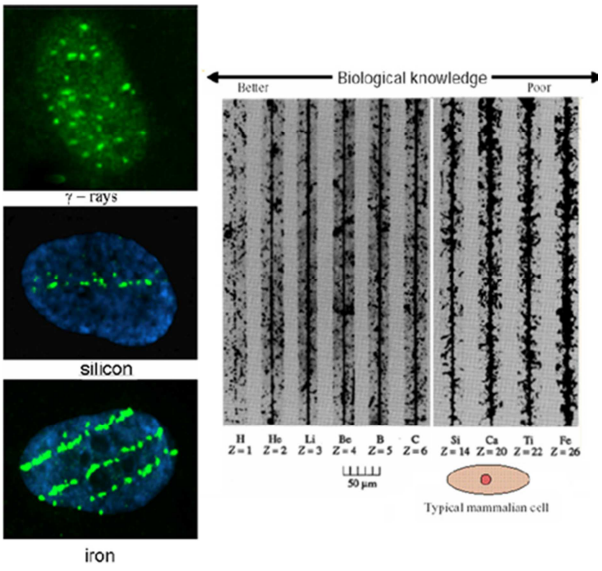

Figure 2. A comparison of particle tracks in nuclear emulsions and human cells labeled for histone γ-H2AX [Cucinotta and Durante, 2006].

Biodosimetry. Although monitoring space radiation exposure for astronauts using physical dosimeters has been routinely performed during space missions, physical dosimeters do not offer information on the details of damage to DNA, cells or tissue and how these damages are repaired in adequate temporal or spatial resolution to assess space radiation health risks accurately. Therefore dosimeters using biological materials to monitor the damage and responses of living cells can offer information that is more relevant to health risks. Passive biodosimeters using dormant biological samples is one practical way to investigate direct biological damage by low-dose and low-dose rate space radiation. The cumulative space radiation-induced DNA damage can then be determined by various biological assays. Because the cells are maintained in a

dormant condition in space with the analyses subsequently performed after returning to Earth, other environmental influences are considered minimal. Valuable assessment can be achieved by comparing the accumulated DNA damage measured in these dormant samples with damage caused by simulated radiation of different qualities, such as low dose rate gamma rays, simulated SEPs, and simulated GCRs on Earth. The data can serve as a reference for other space radiation related studies using LEO, beyond LEO, Sub-orbital, Antarctica Balloon, and ground simulation capabilities. Various real-time radiation biodosimeter concepts have been proposed and tested using genetically modified organisms and micro-photosensors. Most concepts are either to detect direct DNA damage using biomarkers or to measure the activity of early responsive DNA damage sensing and repair proteins, which have been demonstrated to be dose and radiation quality dependent. The exposure dose can be quantified by the intensity of the fluorescent signals in individual cells that will be detected with a photosensor. Even though the sensitivity and specificity of these technologies may be challenging, it potentially provides real-time information directly reflecting the biological impact rather than the particle flux. Biodosimeters are particularly useful for exposures to space radiation which consists of a mixed field of high-energy charged particles, since the biological damage and responses take into account radiation of different qualities.

Space Radiation Induced Biological Effects. Possible detrimental effects of radiation on the human body include cataracts, immune system impairment, cardiovascular problems, infertility and cancer development. At the cellular level radiation induces DNA damage, which needs to be fixed by the cellular repair machinery. To counteract DNA damage, a network of cellular pathways, defined as DNA damage detection and repair response (DDR), accommodates moderate DNA damage by detecting and repairing DNA lesions. These mechanisms consist of, cell cycle regulation, DNA repair, and apoptosis. The basic principles of DDR in prokaryotes and eukaryotes are similar, but significant differences exist in the radiosensitivity among different species and the mechanisms that allow access to the lesions by repair enzymes [Arena et al, 2014]. Plants share many features of chromatin organization and DNA repair with fungi and animals [Arena et al, 2014; Donà and Scheid, 2015]. The biological impact of simulated space radiation on human cells and rodents has been extensively investigated, which is primarily supported by the HRP Space Radiation Element. However, the biological effects of space radiation on other animals, plants, and microorganisms have been less investigated and characterized, even though this knowledge gap may be of equal importance in terms of future interplanetary missions and establishing permanent inhabited bases. These bioregenerative life support systems (see Goal 1) are critical and heavily rely on maintaining healthy interactions among humans, plants and microbial populations [Donà and Scheid, 2015; De Micco et al, 2011]. Effects in plants are significantly influenced by species, cultivar, development stage, tissue architecture and genome organization, as well as radiation features, e.g. quality, dose, and duration of exposure. More and deeper knowledge on the space radiation induced biological effects in non-human and rodent biological systems are critical to ensure the success of the long-term deep space missions. The major biological endpoints to evaluate space radiation effects in these biological systems include survival, produce quality and quantity, reproduction, mutation,

interactions between different species, and the combined effect with other environmental impacts.

Space Radiation Effects on Humans. Space radiation exposure is the primary cause of some detrimental health effects observed in the astronauts. The evidence of cancer risk from ionizing radiation is significant with doses above 50 mSv for low LET radiation such as X-rays or gamma rays as determined by various human epidemiology studies on the survivors of the atomic-bomb explosions in Hiroshima and Nagasaki, and nuclear reactor workers [Cardis et al. 1995, 2007; Huff et al, 2016]. Although a number of astronauts who have flown in space have died of cancer, it is not possible to pinpoint space radiation exposure as the primary cause of these deaths due to the lack of statistical power or proper controls. One of the effects of space radiation exposures on humans is the periodic light flashes experienced by astronauts on their trips to the Moon [Fazio et al. 1970] and in Skylab missions [Pinsky et al. 1975]. On the ground, such light flashes were confirmed to be caused from exposure of the retina to charged particles [Budinger et al. 1972]. Another effect of space radiation exposure is early onset of cataracts [Cucinotta et al. 2001]. Although individuals in the general population are expected to develop cataracts at old age, a group of astronauts that were exposed to higher levels of radiation in the eye were found to develop cataracts at earlier ages than the group receiving lower doses of radiation [Cucinotta et al. 2001]. The third effect of space radiation exposure in astronauts is elevated chromosome aberrations in lymphocytes detected after 3 to 6 month missions on the space station [Yang et al. 1997]. However, the exposure after a typical two-week Space Shuttle mission was so low that no changes in the chromosome aberration frequencies were statistically significant [George et al. 2001].

Relative Biological Effectiveness (RBE). Relative biological effectiveness (RBE) has been widely used to evaluate the impact significance of one type of ionizing radiation relative to another, mostly gamma rays with the same amount of absorbed energy. RBE varies depending on the particles, energies, total energy deposit, flux rate, the relevant biological effects, time-interval post radiation, cell types, cell cycle stages, genetic background, species, and many other factors. There is not sufficient data collected using ground simulated space radiation sources to illustrate a complete RBE values table for human blood samples, mammalian cells, and rodents. Moreover, no data are currently available for other animals, plants, and microorganisms. Experimental data have revealed the following approximate estimated RBE ranges for non-cancer effects in different rodent organ systems: 1 – 5 MeV neutrons, 4 – 8; 5 – 50 MeV neutrons, 2 – 5; heavy ions, 1 – 4; protons > 2 MeV, 1 – 1.5.

Measurement of Cellular Radiation Damage on ISS. There are several experiments specifically designed for investigating the "true" space radiation environment induced DNA damage on ISS. These experiments include one using human cervical carcinoma cells in the Russian MIR space station for 40 days and in the US Space Shuttle for 9 days [Ohnishi et al, 2002], the other using human lymphoblastoid TK6 cells for 4 months [Yatagai et al, 2011]. Furthermore, space radiation has also been reported to induce DNA damage in the lymphocytes of astronauts after long space station flights, as measured by the increased frequency of chromosome aberrations in post-mission samples. In these missions, low but significant DNA

damage has been detected, suggesting space radiation can cause DNA damage. However, the variety of cell types and species are very limited. The dose equivalent rate is relatively low, at about 0.41-0.46 mSv/day on ISS, and most missions are short-term investigations, from several hours to maximum 4 months. The accumulated dose for four months on ISS is about 72 mSv, below the sensitivity threshold of many of biological assays. For instance, fluorescent *in situ* hybridization (FISH), a standard method that has been used for detecting chromosome aberrations in astronauts' blood samples, has a minimum detection dose of about 100 mSv. Therefore, well designed investigations on different biological samples exposed to "true" space radiation for longer duration is needed for a better understanding of space-radiation induced biological effects, as well as human health risks for future space explorations.

Other Indirect Biological Effects. In addition to targeted effects, e.g. DNA damage, indirect (non-targeted) effects of radiation such as bystander effects, adaptive response, and genomic instability have been well reported in human cells and rodent models. Moreover, the space environment presents complex challenges for biological systems where multiple factors may compromise genomic stability. The effects of microgravity and the exposure to toxic compounds or dust particles might indirectly induce biological effects by causing DNA damage and activation of stress responses. These environmental factors may influence the outcome of radiation-induced DNA damage, such as affecting the DNA repair machinery. These indirect or combined biological effects in response to irradiation very likely exist in other species, including plants, other animals, and microorganisms.

SPECIFIC SCIENCE

1. Model Organisms and Example Experiments. For LBLEO missions, the following model organisms and studies are recommended for radiation risk assessment:
    a. Comparison to ground based radiation simulation studies aimed at evaluating the biological effects of true space radiation environment and the effectiveness of ground space radiation simulations is recommended as a high priority. These comparison studies range from human cells to rodent models that have been extensively used for risk assessment on ground.
    b. Rodent models provide the opportunity to investigate physiological or pathological effects induced by combined interplanetary spaceflight environmental factors (including space radiation) in different organs, especially neurological and cardiovascular.
    c. Plant science should graduate from dormant seeds to comparison studies using plants that have been flown on ISS, e.g. *Arabidopsis thaliana*, *Brachypodium distachyon* then to bioregenerative crops and vegetables.
    d. Microorganisms and other small animals that have dormant forms that withstand long-term exposures during deep space flights, possibly with physical track recording.
    e. Biodosimeter development should investigate the use of dormant stages of cells (human, other mammalian cells, and plant cells). Pursue the possibility of real-time biodosimeters.

2. Data Mining and Modeling. Data quality and quantity are critical for further data mining, comparison, and computer modeling.

a. Requires omics and physiological data collected across a wide range of species in response to simulated and/or a true space radiation environment with accurate radiation measurement including information of dose/dose rate/dose equivalent/track/track structure measurement.
b. Requires open access to archived astronauts' and particle-radiotherapy patient data and tissue samples.
c. Requires a GeneLab type data sharing platform.
d. Requires adequate sample size.
e. Review studies on the similarity and uniqueness of biological effects induced by space radiation across a wide range of species.
f. Determination of the variance of radiosensitivity of individuals and individual species and its impact on uncertainty reduction.
g. Studies on interactions and cross-talk among different cell types from different organs.
h. Studies on interactions and cross-talk among different organs if rodent models are available from LBLEO mission.

3. Omics.
   a. The application of 'omics for retrospective analysis of DNA damage and DNA damage responses (DDR) could prove invaluable.
   b. Samples of microorganisms and tissues from higher organisms could prove useful for analysis by deep sequencing, microbiome analysis, proteomics to detect impact on specific DNA sequences, microbial diversity in situ, protein and other macromolecular damage, etc.
   c. Collection and retrospective analysis of a single set of samples across the mission timeframe could reveal the impact of radiation over time while at the same time impacting other goals.

4. Radiation Risks in Space Biology
   a. Decisions will be needed for the selection of most biologically relevant physical parameters (LET, z^2/β^2, track structure, microdosimetry, dose, fluence) to define radiation quality and quantity that, when measured, influence the detrimental effects to key biological systems that have potential impacts on beyond LEO missions.
   b. Radiation induced mutagenesis and genomic instability
   c. Radiation altered host-microbial, and plant-microbial interactions
   d. Radiation altered life span, reproduction, harvest, fertility, and other impacts.
   e. Combined effects of space radiation and other space environmental factors. Is there a spaceflight dose-modifying factor?

5. Radiation Risk Assessment Gaps in Space Biology Prior to LBLEO Missions
   a. Effects on plants, invertebrates, vertebrate and microorganisms using simulated space radiation on the ground.
   b. Combined effects of simulated microgravity and space radiation.
   c. Mechanisms of DNA repair and cellular responses determined from 'omics research relevant to the space radiation environment as indicators of damage and opportunities for countermeasures..
   d. Radiation-sensitivity differences among species and what they reveal about the molecular genetics ('omics) of spaceflight risk.

6. Human radiation biology

a. Develop a systems biology paradigm experimentally, and apply genomics and systems biology analyses to identify individuals at higher risk for combined spaceflight and radiation syndromes.
b. Identify human tissue markers of neurological and cardiovascular radiation damage, including reactions to oxidative stress and countermeasures to oxidative stress.
c. Mine high-LET radiation therapy pathology data for indications of neurological, including cognitive degeneration, or vascular reactions in regions receiving sub-therapeutic doses.

GOAL 9— AN INTEGRATED APPROACH TO ASTROBIOLOGY RELATED INVESTIGATIONS USING DEEP SPACE MISSIONS

The NASA 2015 Astrobiology Strategy presents a set of overarching goals to improve research products designed to provide answers to fundamental questions that relate to how life begins and evolves, the possible existence of life elsewhere in the universe, and the future of life on Earth and beyond. The Strategy recognizes the multi-disciplinary nature of Astrobiology and that within Astrobiology research, individuals bring knowledge that crosses disciplinary boundaries. Astrobiology strategic goals flow down from, and reach back to, the NASA Planetary Science strategic objective to determine the content, origin, and evolution of the Solar System and the potential for life elsewhere [2014 NASA Science Plan]. A set of *Overarching Science Community Goals* are put forth in the Astrobiology Strategy which include: 1) foster interdisciplinary science, 2) enhance NASA missions, 3) promote planetary stewardship, 4) enhance societal interest and relevance, and 5) inspire future generations. These high-level Astrobiology goals can be aligned with many NASA Programs, including NASA Space Biology, and are applicable to the achievement of NASA strategic objectives that reside outside of planetary science. Here, these goals are considered simultaneously for Astrobiology and Space Biology research in the context of Life Beyond Low Earth Orbit. Linking research themes are identified and may point toward more effective means of achieving Astrobiology and Space Biology program objectives, as well as overall NASA agency goals, including the goal to expand the frontiers of knowledge, capability, and opportunity in space [NASA Strategic Plan. 2014].

BACKGROUND

The NASA Space Biology Program and NASA Astrobiology each play individual roles designed to enable the achievement of NASA's overall mission. The Space Biology Program activities, as described in the Space Biology Science Plan 2016-2025, focus on fundamental biological processes that are key to informing knowledge gaps across a continuum of research emphases spanning from Biological Systems through Human Health and on to Human Space Exploration.

As a reminder, official Program Elements are Microbiology, Cell and Molecular Biology, Plant Biology, Animal Biology, and Developmental, Reproductive and Evolutionary Biology. These elements may be compared with those comprising the NASA Astrobiology Strategy. To achieve Space Biology Program goals, current high priority items include animal and plant research on the ISS, cell, microbial and molecular biology on the ISS, as well as a focus on the use of free flyers and microsatellites in support of Space Biology Science [Space Biology Science Plan 2016-2025]. Ultimately, the products the of Space Biology Program research, with its focus on space flight environments, flow upward from the study of biological molecules and microbes, up to human exploration. In comparison, the NASA Astrobiology Strategy, identifies Astrobiology focus topics as follows.

Synopsis of Astrobiology Strategy. The NASA Astrobiology Strategy, identifies Astrobiology focus topics (Table 9.1) along with corresponding key science questions and priority areas of research needed to answer fundamental Astrobiology questions: 1) How does life begin and evolve? 2) Does life exist elsewhere in the universe? and 3) what is the future of life on Earth and beyond?

Coordination. The NASA Space Biology Program and NASA Astrobiology each have specified programmatic goals to enable the achievement of unique objectives. The interdisciplinary nature of Space Biology and Astrobiology stems from the common need for each to utilize researchers, with individual or cross-disciplinary (e.g., physics, chemistry, biology, subdiscipline) expertise to achieve unique program goals. Both Space Biology and Astrobiology at the HQ program level should continue to solicit broad participation by disciplinary and multi-disciplinary scientists and research groups in future funding opportunities, to facilitate cross-disciplinary and potentially interactive research within each program. Common key words can be identified and used to link research needs that are driven by the goal to explore beyond LEO and aligned through knowledge base, methodology developments, and technological implementations. For example the common key words, *Environment and Evolution* can be seen to thematically link Space Biology and Astrobiology. In the context of LBLEO, the themes environment and evolution in Space Biology may be considered to pertain to the study of terrestrial biology IN space and FOR space in the context of human exploration, while in Astrobiology these themes pertain to the potential discovery of non-terrestrial biology in the universe but also to issues of habitability in both cases. To explore these themes beyond LEO, from either a Space Biology or an Astrobiology perspective, scientific tools and technologies are required and represent an area of potential coordination.

Table 9.1 Astrobiology Research Foci and Key Questions from the 2015 NASA Astrobiology Strategy	
Astrobiology Focus Topic	**Areas of Research/Key Questions**
Identifying Abiotic Sources of Organic Compounds	What were the sources, activities, and fates of organic compounds on the prebiotic earth? What is the role of the environment in the production of organic molecules? What is the role of the environment on the stability and accumulation of organic molecules? What constraints can the rock record place on the environments and abiotic reactions of the early earth?
Synthesis and Function of Macromolecules in the Origin of Life	What is the chemistry of macromolecular formation reactions? How does information transmission and chemical evolution occur? What are the chemical alternatives? How and why do they occur? Macromolecular function: how did physicochemical effects develop over time? What are the advanced steps of macromolecular function? What led to macromolecular complexity?
Early Life and Increasing Complexity	Origin and dynamics of evolutionary processes in living systems: theoretical considerations. Fundamental innovations in earliest life. Genomic, metabolic, and ecological attributes of life at the root of the evolutionary tree. Dynamics of the subsequent evolution of life. Common attributes of living systems on earth.
Co-Evolution of Life and the Physical Environment	How does the story of earth—its past, present, and future—inform us about how the climates, atmospheric compositions, interiors, and biospheres of planets can co-evolve? How do the interactions between life and its local environment inform our understanding of biological and geochemical co-evolutionary dynamics? How does our ignorance about microbial life on earth hinder our understanding of the limits of life?
Identifying, Exploring, and Characterizing Environments for Habitability and Biosignatures	How can we assess habitability on different scales? How can we enhance the utility of biosignatures to search for life in the solar system and beyond? How can we identify habitable environments and search for life within the solar system? How can we identify habitable planets and search for life beyond the solar system?
Constructing Habitable Worlds	What are the fundamental ingredients and processes that define a habitable environment? What are the exogenic factors in the formation of a habitable planet? What does earth tell us about general properties of habitability (and what is missing)? What are the processes on other types of planets that could create habitable niches? How does habitability change through time?

Free-flying missions. Technologies for space biology investigations and Astrobiology exploration have facilitated numerous unmanned spaceflight experiments. Key to enabling Space Biology Experiments and Astrobiology Life Detection in environments beyond LEO is the development of automated microfluidic handling and manipulation technologies, along with new automated analytical instrument technologies. ARC has been a lead in the development of miniature fluidic-based analytical platforms for LEO CubeSats in support of the Space Biology and Astrobiology missions listed in Table 9.2. These technology developments have served to

enable the upcoming BioSentinel mission, the first biology experiment beyond low Earth orbit since Apollo and the 1976 Viking Biology Experiments.

Table 9.2 Examples of Space Biology and Astrobiology Free-Flying Mission Technologies with Potential Alignment

Mission	Science	Key Subsystem Technologies
GeneSat-1 LEO	Expression of fluorescent protein in E. coli; microbe population vs. time; 1st biological cubesat payload	No-moving-parts pump for sterile fluids; full-system sterility; 0.2 µm integrated bacteria filters
PharmaSat LEO	Antifungal drug dose response for S. cerevisiae	2 pumps, 11 valves, bubble trap, precision reagent mixing and distribution
O/OREOS LEO	Payload1: B. subtilis survival (6 mo); Payload2: long-term degradation of organic bio-building blocks (1.5 yr); Bus operational 5 years in space	Bubble-free filling of µwells: hydrophobic membranes expel vapor; Perfect sterility (11 months from bio-loading to start)
SporeSat-1 SporeSat-2 LEO	Variable-gravity response of C. richardii fern spores via differential Ca2+ ion channel response	High-complexity electro-opto-mechanical system: illumination + multichannel ion-specific measurement on µcentrifuges
EcAMSat LEO	Antibiotic resistance for pathogenic E. coli in µgravity	Precision reagent dilution, parallel delivery using microfluidic metering
BioSentinel LBLEO	Radiation-induced DNA damage in cells + physical radiation measurements; Integrated optical calibration cells	Monolithic integration: filters, bubble traps, desiccant chambers, valves, check valves, gas expulsion; Fused polycarb fabrication; autoclave-sterilized systems

[1]3U CubeSat, 2006. Exploration Systems Mission Directorate (ESMD), Tech Demo/Fundamental Biology
[2]3U CubeSat, 2009. ESMD, PI-led science mission
[3]3U CubeSat, 2010. Organism/Organic Exposure to Orbital Stresses, Science Mission Directorate (SMD), Astrobiology Small Payloads Prog.
[4]3U CubeSat, 2014. SporeSat-1, SMD Stand Alone Missions of Opportunity; (SporeSat-2 Date TBD)
[5]6U CubeSat, 2016. E. coli Antimicrobial Nanosatellite, NASA CubeSat Launch Initiative (CSLI), Human Exploration and Operations Mission Directorate (HEOMD), Space Life and Physical Sciences Research and Applications Division
[6]6U CubeSat, 2018. NASA Advanced Exploration Systems Program, HEOMD

Investment in Fluid Technologies. One very significant area in which coordination would benefit space investigators across the disciplines is investment in miniaturized fluid technologies to support future space-based investigations be they biomedical, fundamental biology or

astrobiology. Some of these technologies, already under development in astrobiology and space biology labs, will surely have value in terrestrial applications. The versatility of any toolkit that must be taken beyond LEO will be totally dependent on the extent to which each tool can be microminiaturized. A few examples follow. An open tubular ion/liquid chromatograph with pulsed amperometry and UV array detection for identification and chiral separation of amino acids [Liao et al., 2015] has applications in metabolomics, life-search and origin-of-life research. A microfluidic device that contains a monolithic-stack optical instrument and sample reservoir could detect ppb and pptr levels of polycyclic aromatic hydrocarbons (PAHs) in the exploration of outer planets and monitoring of safety aboard interplanetary spacecraft as well as remote or underserved environments on Earth. Nucleic acid extraction and concentration followed by Nanopore-based detection and single-molecule sequencing is already being tested on ISS, as mentioned in other places in this report. A conductivity-based microfluidic ion analyzer in a lab-on-a-chip system that can quantify salts and biomarkers would be a useful tool in physiological monitoring, environmental monitoring and planetary environment characterization. The miniaturization of a nuclear magnetic resonance spectrometer for molecular spectroscopy in remote environments would constitute a major technology coup which NASA investigators could lead [Kim et al., 2012]. Multi-imaging fluorescence/luminescence instruments for exploring seas on icy worlds like Europa and Enceladus, robust to withstand these environments, small enough to carry beyond LEO and solving rigorous telecommunications problems would go a long way toward satisfying numerous broader requirements for interplanetary voyages. Superfluid and supercritical fluid processing are rapidly evolving arts that could be applied to robotic sample separation and analysis for planetary exploration, ISRU and metabolomics if properly miniaturized and energetically feasible. Given the complexities of human and robotic exploration beyond LEO these few examples only begin to address the requirements for serious scientific investigations. It is critical that NASA continues support and development of automated analytical systems for both Space Biology and Astrobiology investigations and missions that extend beyond LEO. Additionally, doing so results in enormous opportunities for NASA to contribute to global technology progress.

Synthetic biology. The widespread availability of gene editing technology (absolutely any gene), especially the CRISPR-Cas9 technology, should revolutionize the way we prepare a living environment for LBLEO, including extraterrestrial settlements. This subject seems to have waned in SMD's astrobiology programs. Breakthroughs facilitating living in space are possible. Gene editing tools are simultaneously applicable to creating built organisms for the built environment for space travelers and to tracking possible events in cellular evolution on the earth (and elsewhere?). Significantly, as a research agency NASA is in a strong position to pursue synthetic biology in the service of LBLEO goals, which are purely scientific, as distinct from biomedical engineering goals and similar potentially controversial applications..

Planetary Protection. Planetary protection research should serve planetary protection practice. Robotic planetary exploration equipment has traditionally been subjected to rigorous preparations to discourage terrestrial microbial passengers. LBLEO research will need to discover acceptable boundaries based on deep perceptions of survival and habitability in order to prepare for the deliberate transport living things – humans and their "friends" to other worlds. Simulated extraterrestrial conditions that do and do not permit the germination of spores have

been explored in a very small number of investigations. Campaigns to characterize a sufficient category of suspect organisms will need to employ more than the small handful of environmental simulators currently available to life scientists in fundamental biology and astrobiology. In this context, planetary protection research (near-term LBLEO) and habitability/ecopoiesis research are both served and need not be in conflict. Resources to date may have been adequate to support essential planetary protection practices, but they have not been adequate to support a continuing and consistent broad planetary protection research activity.

Habitability. Habitability research ranges over all biology disciplines and forms the foundation knowledge applicable to planetary protection, planetary colonization and terraforming/ecopoiesis. In the forthcoming 40-year timeframe research that points to means for modifying planetary environments and modifying organisms to bring their characteristics into compatibility will experience a rising emphasis. This will require the combined open-minded imagination characteristic of NIAC Fellows, environmental understanding characteristic of planetary scientists, and tools of the fundamental biologist. This 40+-year outlook needs wider attention and should not be limited to NIAC calls and giant rocket development. This calls for research on Earth using simulated and modified simulated planetary/lunar environments, extremophiles and genetically modified extremophiles. The movement of such simulator-based research into the LBLEO environment has intriguing possibilities for including the reduced-gravity and increased-radiation conditions, which are otherwise unavailable in terrestrial simulators. In situ resource utilization (ISRU) is also a component of habitability research. There are numerous Space Biology opportunities associated with ISRU. There has been brief consideration of bio-mining the moon, orbital planetary atmospheric resource mining, bio-mining resources for printable electronics and similar undertakings, some of which have been partially sponsored by NASA Innovative Advanced Concepts (NIAC). The roles of Space Biology and Astrobiology in ISRU, a very significant component of beyond LEO planning could benefit from increased attention.

SPECIFIC SCIENCE AND TECHNOLOGY

1. NASA workshops to bring scientists and engineers engaged in HEOMD and SMD funded technology development, that may align with both Space Biology and Astrobiology science needs, should be supported.
2. Integrate CubeSat technology into combined Space Life Science and Astrobiology experiments beyond LEO.
3. Integrate Nanopore sequencing technology broadly across NASA life science and astrobiology research programs.
4. Apply methods of synthetic biology broadly to origin-of-life research and novel life forms for the built, beyond-LEO environment.
5. Utilize the fruits of planetary research and exobiology to inform ISRU opportunities and potential practices.
6. Terrestrial research applied to the refinement of concepts for planetary protection.
7. Integration of habitability research across administrative boundaries.
8. Invigorate planetary protection research for the purposes of establishing future levels of rigor in planetary protection practice and for anticipating the deliberate transport of organisms to extraterrestrial bodies.

Appendix 1 Acronyms

AES	Advanced Exploration Systems (under HEOMD)
ARC	Ames Research Center
ASGSR	American Society for Gravitational and Space Research
CASIS	Center for the Advancement of Science in Space
CNS	Central Nervous System
CSA	Canadian Space Agency
DLR	German Space Agency
EM	Exploration Mission (using Orion capsule)
ESA	European Space Agency
HEOMD	Human Exploration & Operations Mission Directorate
HRP	Human Research Program (under HEOMD)
ISS	International Space Station
JAXA	Japanese Space Agency
JSC	Johnson Space Center
LBLEO	Life Beyond Low Earth Orbit
LEO	Low Earth Orbit
NASA	National Aeronautics and Space Administration
NIAC	NASA Institute for Advanced Concepts
PSD	Planetary Sciences Division (under SMD)
RSA	Russian Space Agency
SLPS	Space Life and Physical Sciences (under HEOMD)
SLS	Space Launch System
SMD	Science Mission Directorate
STMD	Space Technology Mission Directorate
STS	Space Transportation System (space shuttle)
SWG	Science Working Group

Appendix 2. References, Alphabetical for each Goal

GOAL 2 References

Barrila, J., Wilson, J.W., Soni, A., Yang, J., Ott, C.M., Nickerson, C.A. (2016). Using spaceflight and spaceflight analogue culture for novel mechanistic insight into *Salmonella* pathogenesis, in *Effect of Spaceflight and Analogue Culture on Human and Microbial Cells: Novel Insights into Disease Mechanisms.* Nickerson, C.A., Ott, C.M., Pellis, N., (Eds). Springer Publishers, New York, pp. 209-235.

Castro, S.L., Niesel, D. W., Barrila, J., Ott, C.M. (2016). Spaceflight and spaceflight analogue induced responses in Gram positive bacteria. In *Effect of Spaceflight and Spaceflight Analogue Culture on Human and Microbial Cells: Novel Insights into Disease Mechanisms* (CA Nickerson, CM Ott, N Pellis, eds.). Springer Publishers, New York, pp. 283-296.

Crabbé, A., Barrila, J., Ott, C.M., Nickerson, C. A. (2016). Outpacing infectious disease: Mimicking the host-pathogen microenvironment, in *Effect of Spaceflight and Analogue Culture on Human and Microbial Cells: Novel Insights into Disease Mechanisms.* Nickerson, C.A., Ott, C.M., Pellis, N., (Eds). Springer Publishers, New York, pp. 93-119.

Crabbé, A., Ledesma Barrera, A., Ott, C.M., C.A. Nickerson, C.A. (2016). Response of *Pseudomonas aeruginosa* to spaceflight and spaceflight analogue culture: Implications for astronaut health and the clinic, in *Effect of Spaceflight and Analogue Culture on Human and Microbial Cells: Novel Insights into Disease Mechanisms.* Nickerson, C.A., Ott, C.M., Pellis, N., (Eds). Springer Publishers, New York, pp. 237-57.

Nickerson, C.A., Ott, C.M., Pellis, N., (Eds) (2016). *Effect of Spaceflight and Analogue Culture on Human and Microbial Cells: Novel Insights into Disease Mechanisms.* Kluwer Academic/Plenum Press, New York, 310 pp.

Nickerson, C.A., Ott, C. M. (2016). Biomedical advances in three dimensions: An overview of human cellular studies in space and spaceflight analogues, in *Effect of Spaceflight and Analogue Culture on Human and Microbial Cells: Novel Insights into Disease Mechanisms,* Nickerson, C.A., Ott, C.M., Pellis, N., (Eds). Springer Publishers, New York. pp. 83-92.

Ott, C.M., Crabbé, A., Wilson, J.W., Barrila, J.B, Nickerson, C. A. (2011). Microbial Stress: Spaceflight-induced alterations in microbial virulence and infectious disease risks for the crew, in *Stress Challenges and Immunity in Space,* Ed. A. Chouker.. Springer, New York, pp 203-225

Singh, R., AC Matin, A.C. (2016). Cellular response of *Escherichia coli* to microgravity and microgravity analogue culture. In *Effect of Spaceflight and Spaceflight Analogue Culture on Human and Microbial Cells: Novel Insights into Disease Mechanisms* (Nickerson, C. A., Ott, C. M., Pellis, N., eds.). Springer Publishers, New York, pp. 259-282.

GOAL 3 References

Barrila, J, Ott, C.M., LeBlanc, C, Mehta, S.K., Nelman-Gonzales, M, Crabbé, A., Stafford, P., Pierson, D., Nickerson, C.A. (2016). Spaceflight modulates gene expression in the whole blood of astronauts. npj Microgravity. 2, 16039; doi:10.1038/npjmgrav.2016.39

Chouker, A. (Ed.) (2011). *Stress Challenges and Immunity in Space*, Springer, 461 pp.

Crucian, B., Stowe, R.P., Mehta, S., Quiriarte, H., Pierson, D., Sams, C. (2015). Alterations in adaptive immunity persist during long-duration spaceflight. npj Microgravity. 1:15013

Crucian, B, A Babiak-Vazquez, A., S Johnston, S., DL Pierson, D.L., Ott, C.M., Sams, C. (2016). Incidence of clinical symptoms during long-duration orbital spaceflight. Int J Gen Med 9 383–391.

Nickerson, C.A., Ott, C. M. (2016). Biomedical advances in three dimensions: An overview of human cellular studies in space and spaceflight analogues, in *Effect of Spaceflight and Analogue Culture on Human and Microbial Cells: Novel Insights into Disease Mechanisms,* Nickerson, C.A., Ott, C.M., Pellis, N., (Eds). Springer Publishers, New York. pp. 83-92.

Tauber, S., Yi, B., Choukèr, A., Ullrich, O. (2016).Use of in vitro cell culture models to understand the cellular and molecular basis of immune dysfunction during spaceflight, in *Effect of Spaceflight and Analogue Culture on Human and Microbial Cells: Novel Insights into Disease Mechanisms.* Nickerson, C.A., Ott, C.M., Pellis, N., (Eds). Springer Publishers, New York, pp. 121-130.

Yi, B., Crucian, B., Tauber, S., Ulrich, O, Choukèr, A.(2016) Immune dysfunction in spaceflight - An integrative view, in *Effect of Spaceflight and Analogue Culture on Human and Microbial Cells: Novel Insights into Disease Mechanisms.* Nickerson, C.A., Ott, C.M., Pellis, N., (Eds). Springer Publishers, New York, pp. 61-82.

GOAL 4 References

Caiozzo, V.J., Haddad, F., Baker., M.J., Herrick, R.E., Prietto, N., Baldwin, K.M. (1996). Microgravity-induced transformations of myosin isoforms and contractile properties of skeletal muscle. Journal of Applied Physiology. 81(1): 123-32. PMID 8828554.

Dallas, S. L., Prideaux, M., Bonewald, L. F. (2013). The Osteocyte: An Endocrine Cell … and More. Endocrine Reviews 34, 658–690, https://doi.org/10.1210/er.2012-1026.

Lee, S.M., Shakelford, L.C., Smith, S.M., Guilliams, M.E., Loehr, J.A., Shepherd, B., Laughlin, M.S., Chauvin, J., Hagan, R.D.. (2004). Lean tissue mass and muscle strength: Does resistive exercise prevent space flight deconditioning? Med Sci Sports Exerc. 36 (5): S272. doi:10.1249/00005768-200405001-01304.

Reschke, M.F., Bloomberg J. J., Paloski, W. H., Harm, D.L. Parker, D.E. (1994). Physiologic adaptation to space flight – neurophysiologic aspects: sensory and motor function, in Space Physiology and Medicine, A. E. Nicogossian, C. L. Huntoon and S. L. Pool, eds. Lea and Febiger, pp. 261-285.

Riley, D.A., Thompson, J.L., Krippendorf, B.B., Slocum, G.R. (1995). "Review of spaceflight and hindlimb suspension unloading induced sarcomere damage and repair" (PDF). Basic and Applied Myology. 5 (2): 139–45. PMID 11539271.

Roy, R.R., Baldwin, K.M., Edgerton, V.R. (1996). Response of the neuromuscular unit to spaceflight: what has been learned from the rat model . Exercise and sport sciences reviews. 24: 399–425. PMID 8744257. doi:10.1249/00003677-199600240-00015.

Schnyder, S., Handschin, C. (2015). Skeletal muscle as an endocrine organ: PGC-1α, myokines and exercise. Bone 80, 115-125. doi: 10.1016/j.bone.2015.02.008.

Shiba, N., Matsuse, H., Takano, Y., Yoshimitsu, K., Omoto M., Hashida R., Tagawa Y., Inada T., Yamada S., Ohshima H. (2015) .Electrically stimulated antagonist muscle contraction increased muscle mass and bone mineral density of one astronaut - Initial verification on the International Space Station. PLOS ONE. 2015 August 21; 10(8): e0134736. DOI: 10.1371/journal.pone.0134736. PMID: 26296204.

Sibonga, J. (2017a). Risk of Bone Fracture Due to Spaceflight-Induced Changes to Bone. https://humanresearchroadmap.nasa.gov/risks/risk.aspx?i=77.

Sibonga, J. (2017b). Risk of Early Onset Osteoporosis Due to Space Flight. https://humanresearchroadmap.nasa.gov/risks/risk.aspx?i=90.

Sibonga, J. (2017c). Risk of Renal Stone Formation https://humanresearchroadmap.nasa.gov/risks/risk.aspx?i=81.

Young, L.R . (1984). Perception of the Body in Space; Mechanisms; Handbook of Physiology, Sec 1, The Nervous System , Vol III Part 2, ed by Darian Smith, American Physiological Society, Bethesda, Oxford University Press.

Young, L. R., Oman, C.M., Watt, D.G.D., Money, K. E., Lichtenberg, B. K. (1984). Spatial Orientation in Weightlessness and readaptation to Earth's Gravity. Science, 225, 205-208.

GOAL 5 References

Anzai, T., Frey, M. A., Nogami, A. (2014). Cardiac arrhythmias during long-duration spaceflights. Journal of Arrhythmia 30, 139-49. https://doi.org/10.1016/j.joa.2013.07.009.

Bier, E., Bodmer, R. (2004). Drosophila, an emerging model for cardiac disease. Gene 342, 1-11.

BioOptics World (2017). Posted by Lee Dubay. Handheld laser scanner to diagnose cardiovascilar diseases instantly. http://www.bioopticswprld.com/articles/2017/07/ handheld-laser-scanner-to-diagnose-cardiovascilar-diseases-instanly.html.

Chang, C.C., Boland, E.D., Williams, S.K., Hoying, J.B. (2011). "Direct-Write Bioprinting Three-Dimensional Biohybrid Systems for Future Regenerative Therapies."Journal of Biomedical Materials Research. Part B 98(1):160-170).

Delp, M. D., Charvat, J. M., Limoli, C. L., Globus, R. K., Ghosh, P. (2016). Apollo Lunar Astronauts Show Higher Cardiovascular Disease Mortality: Possible Deep Space Radiation Effects on the Vascular Endothelium. Scientific Reports 6 Article number: 29901 doi:10.1038/srep29901

Doarn, C.R., Pantalos, G., Strangman, G., Broderick, T.J. (2016). Surgical Capabilities for Exploration and Colonization Space Flight – An Exploratory Symposium. NASA Technical Publication TP-2016219281, NASA Johnson Space Center, November. https://ston.jsc.nasa.gov/collections/TRS/_techrep/TP-2016-219281.pdf.

Hayden, J., Pantalos, G., Burgess, J., Antaki, J. (2013). Creating a Hermetically Sealed, Fluid-filled Surgical Enclosure to Perform Procedures in Reduced Gravity. Aviat Space Environ Med; 84:12981303.

Mednieks. M., Khatri, A., Rubenstein, R., Burleson, J.A., Hand, A.R. (2014). Microgravity alters the expression of salivary proteins. 2014. Oral Health Dent Manag 13(2): 211-216. PMID: 24984624.

National Aeronautics and Space Adminsitration (NASA) (2016). Chapter X In SLPSRA Integrated Research Plan Space Biology Plan 2016-2025. National Academy of Medicine. 2016. Review of NASA's evidence report on human health risks: 2015 Letter report. National Academies Press, Washington. DOI: 10.17226/21844

Neely, G.G., Kuba, K., Cammarato, A., Isobe, K., Amann, S., Zhang, L., Murata, M., Elmen, L., Gupta, V., Arora, S., Sarangi, R., Dan, D., Fujisawa, S., Usami, T., Xia, C.P., Keene, A.C., Alayari, N.N., Yamakawa, H., Elling, U., Berger, C., Novatchkova, M., Koglgruber, R., Fukuda, K., Nishina, H., Isobe, M., Pospisilik, J.A., Imai, Y., Pfeufer, A., Hicks, A.A., Pramstaller, P.P., Subramaniam, S., Kimura, A., Ocorr, K., Bodmer, R., Penninger, J.M. (2010). A global in vivo *Drosophila* RNAi screen identifies NOT3 as a conserved regulator of heart function. Cell 141(1):142-53.doi 10.1016/j.cell.2010.02.023. PMID: 20371351.

Ocorr, K., Akasaka, T., Bodmer, R. (2007). Age-related Cardiac Disease Model of Drosophila. Mech Ageing Dev. 128(1):112-116

Pantalos, G.M., Bennett, T.E., Sharp, M.K., Woodruff, S.J., O'Leary, S.D., Gillars, K.J., Schurfranz, T., Everett, S.D., Lemon, M., Schwartz, J. (2005): The effect of gravitational acceleration on cardiac diastolic function: A biofluid mechanical perspective with initial results. Cur Pharm Biotech, 6(4): 331-341.

Sofronova, S.I., Tarasova, O.S., Gaynullina, D., Borzykh, A.A., Behnke, B.J., Stabley, J.N., McCullough, D.J., Maraj, J.J., Hanna, M., Muller-Delp, J.M., Vinogradova, O.L., Delp, M.D. (2015). Spaceflight on the Bion-M1 biosatellite alters cerebral artery vasomotor and mechanical properties in mice. *J Appl Physiol* 118(7): 830-838. PMID: 25593287.

Ugur, B., Chen, K., Bellen, H.J. (2016). Drosophila tools and assays for the study of human diseases. Disease Models and Mechanisms 9 :235-244.

Van der Harst, P., Van Setten, J. et al 157 authors (2016). 52 Genetic loci influencing myocardial mass. J Am College of Cardiology, 68(13):1435-1448. doi 10.1016/j.jacc.2016.07.729.PMID:27659466.

Vener, J.M., Pantalos, G.M., Miller, S.C. (2004); Indices of cardiac atrophy and myocyte apoptosis after 14 Days of hindlimb unloading in rats. Med & Sci in Sports & Exercise 36(5), p. S271-S272.

Yan, X., Sasi, S.P., Gee, H., Lee, J., Yang, Y., et al. (2015) Cardiovascular risks associated with low dose ionizing particle radiation. PLOS ONE 10(11): e0142764. https://doi.org/10.1371/journal.pone.0142764

GOAL 6 References:

Antsiferova, L.I., Shlyk, G.G., Ignatenko, A.V. (2000). Effect of microgravity on the implementation of conditioned reflex skills of rhesus monkeys. J Gravit Physiol. 7(1):S95-8.

Balaban, P.M., Malyshev, A.Y., Ierusalimsky, V.N., Aseyev, N., Korshunova, T.A., Bravarenko, N.I., Lemak, M.S., Roshchin, M., Zakharov, I.S., Popova, Y., Boyle, R. (2011). Functional changes in the snail statocyst system elicited by microgravity. PLoS One. 6(3):e17710.

Baulch, J.E., Craver, B.M., Tran, K.K., Yu, L., Chmielewski, N., Allen, B., Limoli, C.L. (2015). Persistent oxidative stress in human neural stem cells exposed to low fluences of charged particles. Redox Biology 5: 24–32.

Benguría, A., Grande, E., de Juan, E., Ugalde, C., Miquel, J., Garesse, R., Marco, R. (1996). Microgravity effects on *Drosophila melanogaster* behavior and aging. Implications of the IML-2 experiment. J Biotechnol. 47(2-3): 191-201.

Britten, R. A., Davis, L. K., Jewell, J. S., Miller, V. D., Hadley, M. M., Sanford, L. D., Machida, M., Lonart, G. (2014). Exposure to Mission Relevant Doses of 1 GeV/Nucleon ^{56}Fe Particles Leads to Impairment of Attentional Set-Shifting Performance in Socially Mature Rats. Radiat. Res. 182, 292– 298.

Britten, R. A., Davis, L. K., Johnson, A. M., Keeney, S., Siegel, A., Sanford, L. D., Singletary, S. J. Lonart, G. (2012). Low (20 cGy) Doses of 1 GeV/u ^{56}Fe-Particle Radiation Lead to a Persistent Reduction in the Spatial Learning Ability of Rats. Radiat. Res. 177: 146–151.

Britten, R. A., Mitchell, S., Johnson, A. M., Singletary, S. J., Keeney, S. K., Nyalwidhe, J. O., Karbassi, I. D., Lonart, G., Sanford, L. D., Drake, R. R. (2010). The identification of serum biomarkers of high-LET radiation exposure and biological sequelae. Health Phys. 98 (2):196-203.

Cai, Y.L., Ma, W.L., Li, M., Ouyang, R.Y., Hu, L., Sun, Y. L. (2005) Behavioral changes of rats under rotation stimulation. Space Med Med Eng (Beijing). 18(2): 98-101.

Canu, M.H., Darnaudéry, M., Falempin, M., Maccari, S., Viltart, O. (2007). Effect of hindlimb unloading on motor activity in adult rats: impact of prenatal stress. Behav Neurosci. 121(1):177-85.

Cassady, K., Koppelmans, V., Reuter-Lorenz, P., De Dios, Y., Gadd, N., Wood, S., Castenada, R.R., Kofman, I., Bloomberg, J., Mulavara, A., Seidler, R. (2016). Effects of a spaceflight analog environment on brain connectivity and behavior. Neuroimage. 141:18-30.

Chakraborti, A., Allen, A., Allen, B., Rosi, S., Fike, J.R. (2012). Cranial irradiation alters dendritic spine density and morphology in the hippocampus. PLoS One. 7(7):e40844. PMID: 22815839.

Chang, P. Y., Doppalapudi, R., Bakke, J., Wang, A., Menda, S., Davis, Z. (2010). Biological impact of low dose-rate simulated solar particle event radiation in vivo. Radiat Environ Biophys. (2010) 49(3), 379-388.

Cherry, J.D., Liu, B., Frost, J.L., Lemere, C.A., Williams, J.P., Olschowka, J., O'Banion, M.K. (2012). Galactic Cosmic Radiation Leads to Cognitive Impairment and Increased Ab Plaque Accumulation in a Mouse Model of Alzheimer's Disease. PLoS ONE 7(12): e53275.

Cucinotta, F.A., Alp, M., Sulzman, F.M. Wang, M. (2014). Space Radiation Risks to the Central Nervous System. Life Sciences in Space Research 2, 54–69.

Cucinotta, F.A., Nikjoo, H., Goodhead, D.T., Wilson, J.W. (1998). Comment on the Effects of Delta-Rays on the Number of Particle-Track Transversals Per Cell in Laboratory and Space Exposures. Radiation Research.150(1):115-119.

Curtis, S.B., Vazquez, M.E., Wilson, J.W., Atwell, W., Kim, M.H. (2000). Cosmic ray hits in the central nervous system at solar maximum. Adv Space Res. 25(10):2035-2040.

Davis, C. M., DeCicco-Skinner, K. L., Roma, P. G. Hienz, R. D. (2014). Individual Differences in Attentional Deficits and Dopaminergic Protein Levels following Exposure to Proton Radiation. Radiat. Res. 181, 258–271.

Francia, N., Santucci, D., Aloe, L., Alleva, E. (2004). Neurobehavioral coping to altered gravity: endogenous responses of neurotrophins. Prog Brain Res. 146:185-94.

Giedzinski, E., Rola, R., Fike, J.R., Limoli, C.L. (2005). Efficient production of reactive oxygen species in neural precursor cells after exposure to 250 MeV protons. Radiat Res. 2005; 164(4 Part 2):540-544.

Greene-Schloesser, D., Robbins, M.E., Peiffer, A.M., Shaw, E.G., Wheeler, K.T. Chan, M.D. (2012). Radiation-Induced Brain Injury: A Review. Front Oncol. 2: 73.

Haerich, P., Nelson, G.A., Pecaut, M.J. (2005). HZE radiation and dopaminergic modification of startle and prepulse inhibition in mice. Physiol Behav. 86(1-2):103-10.

Horn, E., Agricola, H., Böser, S., Förster, S., Kämper, G., Riewe, P., Sebastian, C. (2002). Crickets in Space: Morphological, Physiological and Behavioral Alterations Induced by Space Flight and Hypergravity. Adv. Space Res. 30: 819-828.

Hosoi, Y., Fujino, M., Hirai, M., Mizuno, R., Ijiri, K., Suzuki, T.(2003). Behavior of Medaka fish under distributed gravity. Biol Sci Space. 17(3): 238-9.
Ijiri, K. (2004). Ten years after Medaka fish mated and laid eggs in space and further preparation for the life-cycle experiment on ISS. Biol Sci Space. 18(3): 138-9.

Il'in, E.A., Smirnov, I.A., Soldatov, P.E., Orlov, O.I. (2009). Gerbil experiment in the flight of spacecraft "Foton-M3". Aviakosm Ekolog Med. 43(4):21-5.

Iqbal, J., Li, W., Hasan, M., Juan Li, Y., Ullah, K., Yun, W., Awan, U., Qing, H., Deng, Y. (2014). Distortion of homeostatic signaling proteins by simulated microgravity in rat hypothalamus: A(16) O/(18) O-labeled comparative integrated proteomic approach. Proteomics. 14: 262-73.

Ishida, Y., Ohmachi, Y., Nakata, Y., Hiraoka, T., Hamano, T., Fushiki, S. Ogiu, T. (2006). Dose-Response and Large Relative Biological Effectiveness of Fast Neutrons with Regard to Mouse Fetal Cerebral Apoptosis. J. Radiat. Res. 47: 41-47.

Izumi-Kurotani, A., Mogami, Y., Okuno, M., Yamashita, M. (1997). Frog experiment onboard space station Mir. Adv Space Biol Med. 6: 193-211.

Jenrow, K. A., Brown, S. L., Lapanowski, K., Naei, H., Kolozsvary, A., Kim, J. H. (2013). Selective Inhibition of Microglia-Mediated Neuroinflammation Mitigates Radiation- Induced Cognitive Impairment. Radiat. Res. 179, 549– 556.

Kempf, S.J., Buratovic, S., von Toerne, C., Moertl, S., Stenerlöw, B., Hauck, S.M., Atkinson, M.J., Eriksson, P., Tapio, S. (2014a). Ionising Radiation Immediately Impairs Synaptic Plasticity-Associated Cytoskeletal Signalling Pathways in HT22 Cells and in Mouse Brain: An In Vitro/In Vivo Comparison Study. PLoS ONE 9(10): e110464. doi:10.1371/journal.pone. 0110464.

Khan, S., Tariq, M., Perrott, J., Brumbaugh, C., Kim, Y., Shabbir, M., Ramesh, G., Pourmand, N. (2013). Distinctive microRNA expression signatures in proton-irradiated mice. Mol Cell Biochem 382:225–235.

Kumar, M., Haridas, S., Trivedi, R., Khushu, S., Manda, K. (2013). Early cognitive changes due to whole body γ-irradiation: A behavioral and diffusion tensor imaging study in mice. Experimental Neurology 248:360–368.

Lee. S-H., Dudok, B., Parihar, V., Jung, K-M., Zöldi, M., Kang, Y-J., Maroso, M., Alexander, A., Nelson, G., Piomelli, D., Katona, I., Limoli, C., Soltesz, I. (2016). Neurophysiology of space

travel: energetic solar particles cause cell type-specific plasticity of neurotransmission. Brain Struct Funct doi:10.1007/s00429-016-1345-3.

Lim, Y-B., Pyun, B-J., Lee, H-J., Jeon, S-R., Jin, Y.B. Lee, Y-S. (2011). Proteomic identification of radiation response markers in mouse intestine and brain Proteomics 11: 1254–1263.

Lonart, G., Parris, B., Johnson, A.M., Miles, S., Sanford, L.D., Singletary, S.J., Britten, R.A. (2012). Executive function in rats is impaired by low (20 cGy) doses of 1 GeV/u 56Fe particles. Radiat Res. 178: 289-94.

Lowe, X.R., Bhattacharya, S., Marchetti, F., Wyrobek, A.J. (2009). Early brain response to low-dose radiation exposure involves molecular networks and pathways associated with cognitive function, advanced aging and Alzheimer's disease. Radiat Res 171: 53-65.

Mao, X. W., Favre, C. J., Fike, J. R., Kubinova, L., Anderson, E. R., Campbell-Beachler M., Jones, T., Smith, A., Rightnar, S., Nelson, G. A., (2010). High-LET radiation-induced response of microvessels in the hippocampus. Radiat Res. 2010 Apr;173(4):486-93.

Mitani, K., Horii, A., Kubo, T. (2004). Impaired spatial learning after hypergravity exposure in rats. Brain Res Cogn Brain Res. 22(1):94-100.

National Council on Radiation Protection and Measurements NCRP (2006), National Council on Radiation Protection and Measurements. Information Needed to Make Radiation Protection Recommendations for Space Missions Beyond Low-Earth Orbit. Report 153, Bethesda, MD.

National Council on Radiation Protection and Measurements NCRP (2014). Radiation Protection for Space Activities: Supplememnt to Previous Recommendations. NCRP Commentary #23. National Council on Radiation Protection and Measurements. Bethesda, MD.

Nelson, G.A. (2009) Neurological Effects of Space Radiation Gravitational and Space Biology 22(2) 33-38.

Nelson, G.A., Schubert, W.W., Kazarians, G.A., Richards, G.F. (1994). Development and Chromosome Mechanics in Nematodes: Results from IML-1. Advances in Space Research 14(8): 209-214.

Norbury, J.W., Slaba, T.C. (2014). Space radiation accelerator experiments – The role of neutrons and light ions. Life Sciences in Space Research 3: 90–94.

Obenaus, A., Mickley, G.A, Bogo, V., West, B.R., Raber, J., (2012). Behavioral and Neurophysiological Consequences of Radiation Exposure. In Medical Consequences of Radiological and Nuclear Weapons. Sr. Editor Mickelson, A.B. Office of the Surgeon General, Borden Institute, Fort Detrick, MD. Chapter 7. pp127 - 169.

Oosterveld, W.J., de Jong, H.A. (1987). The effect of weightlessness on the flight behavior of pigeons with canal lesions. Aviat Space Environ Med. 58(9 Pt 2): A250-2.

Otake, M., Schull, W.J. (1998). Radiation-related brain damage and growth retardation among the prenatally exposed atomic bomb survivors. Int J Radiat Biol. Aug;74(2):159-71.

Parihar, V.K., Allen, B., Tran, K.K., Macaraeg, T.G., Chu, E.M., Kwok, S.F., Chmielewski, N.N., Craver, B.M., Baulch, J.E., Acharya, M.M., Cucinotta, F.A., Limoli, C.L. (2015a). What happens to your brain on the way to Mars. Sci. Adv. 1:e1400256.

Parihar, V.K., Pasha, J., Tran, K.K., Craver, B.M., Acharya, M.M., Limoli, C.L. (2015b). Persistent changes in neuronal structure and synaptic plasticity caused by proton irradiation. Brain Struct Funct 220:1161–1171.

Porseva, V.V., Shilkin, V.V., Strelkov, A.A., Krasnov, I.B., Masliukov, P.M. (2017). Changes in the Neurochemical Composition of Motor Neurons of the Spinal Cord in Mice under Conditions of Space Flight. Bull Exp Biol Med. 162(3): 336-339.

Poulose, S.M., Bielinski, D.F., Carrihill-Knoll, K., Rabin, B.M., Shukitt-Hale, B. (2011). Exposure to ^{16}O-particle radiation causes aging-like decrements in rats through increased oxidative stress, inflammation and loss of autophagy. Radiat Res 176: 761-769.

Qiao, L., Luo, S., Liu, Y., Li, X., Wang, G., Huang, Z. (2013). Reproductive and locomotory capacities of Caenorhabditis elegans were not affected by simulated variable gravities and spaceflight during the Shenzhou-8 mission. Astrobiology 13(7): 617-25.

Raber, J., Allen, A.R., Rosi, S., Sharma, S., Dayger, C., Davis, M.J., Fike, J.R. (2013). Effects of whole body ^{56}Fe radiation on contextual freezing and Arc-positive cells in the dentate gyrus. Behav Brain Res. 246:162-7.

Raber, J., Rosi, S., Chakraborti, A., Fishman, K., Dayger, C., Davis, M.J., Villasana, L., Fike, J.R. (2011). Effects of ^{56}Fe-particle cranial radiation on hippocampus-dependent cognition depend on the salience of the environmental stimuli. Radiat. Res 176(4):521-6.

Rabin, B.M., Shukitt-Hale, B., Joseph, J.A., Carrihill-Knoll, K.L., Carey, A.N., Cheng, V. (2007). Relative effectiveness of different particles and energies in disrupting behavioral performance. Rad. Env. Biophys. 46: 173-177.

Rahmann, H., Anken, R.H. (2000). Gravitational Neurobiology of Fish. Adv. Space Res. 25(10): 1985-1995.

Rice, O.V., Grande, A.V., Dehktyar, N., Bruneus, M., Robinson, J.K., Gatley, S. J. (2009). Long-term effects of irradiation with iron-56 particles on the nigrostriatal dopamine system. Radiat Environ Biophys 48(2) 215-225.

Rola, R., Otsuka, S., Obenaus, A., Nelson, G.A., Limoli, C.L., VandenBerg, S.R., Fike, J.R. (2004). Indicators of hippocampal neurogenesis are altered by ^{56}Fe-particle irradiation in a dose-dependent manner. Radiat Res. 162(4):442-446.

Schultheiss, T.E., Kun, L.E., Ang, K.K., Stephens, L.C. (1995). Radiation response of the central nervous system. Int. J. Radiat. Onc. Biol. Phys.. 31(5):1093-1112.

Shimomura-Umemura, S., Ijiri, K. (2006). Effect of hypergravity on expression of the immediate early gene, c-fos, in central nervous system of medaka (Oryzias latipes) Advances in Space Research 38:1082–1088.

Shukitt-Hale, B., Casadesus, G., McEwen, J.J., Rabin, B.M., Joseph, J.A. (2000). Spatial learning and memory deficits induced by exposure to iron-56-particle radiation. Radiat Res.154(1):28-33.

Slaba, T.C., Blattnig, S.R., Norbury, J.W., Rusek, A., La Tessa, C., Walker, S.A. (2015). GCR Simulator Reference Field and a Spectral Approach for Laboratory Simulation. NASA/TP-2015-218698. National Aeronautics and Space Administration Langley Research Center, Hampton, Virginia.

Sokolova, I. V., Schneider, C. J., Bezaire, M., Soltesz, I., Vlkolinsky, R., Nelson, G.A., (2015). Proton Radiation Alters Intrinsic and Synaptic Properties of CA1 Pyramidal Neurons of the Hippocampus. Radiat. Res. 183: 208–218.

Space.com (2012). http://www.space.com/6124-astronauts-watch-space-spiders-weave-wild-webs.html [4/30/2012 2:49:23 PM].

Strangman, G. E., Sipes, W., Beven, G. (2014). Human cognitive performance in spaceflight and analogue environments. Aviation, Space, and Environmental Medicine, 85(10), 1033–1048.

Tanaka, Y., Nakano, J., Hamaue, Y., Sekino, Y., Sakamoto, J., Kataoka, H., Okita, M. (2013). Hindlimb suspension does not influence mechanical sensitivity, epidermal thickness, and peripheral nerve density in the glabrous skin of the rat hind paw. Physiol Res. 62(1):119-23.

Tofilon, P.J., Fike, J.R., (2000). The radioresponse of the central nervous system: a dynamic process. Radiat Res. 2000; 153(4):357-370.

Tseng, B.P., Giedzinski, E., Izadi, A., Suarez, T., Lan, M.L., Tran, K.K., Acharya, M.M., Nelson GA, Raber J, Parihar VK, Limoli CL. (2014). Functional consequences of radiation-induced oxidative stress in cultured neural stem cells and the brain exposed to charged particle irradiation. Antioxid Redox Signal. 20:1410-22.

Vazquez, M.E., (1998). Neurobiological problems in long-term deep space flights. Adv. Space Res. 22(2): 171-183.

Villasana, L., Rosenberg, J., Raber, J.(2010). Sex-dependent effects of ^{56}Fe irradiation on contextual fear conditioning in C57BL/6J mice. Hippocampus 20: 19-23.

Vlkolinský, R., Krucker, T., Nelson, G.A., Obenaus, A. (2008). $^{(56)}$Fe-particle radiation reduces neuronal output and attenuates lipopolysaccharide-induced inhibition of long-term potentiation in the mouse hippocampus. Radiat Res 169(5):523-530, 2008.

Vlkolinský, R., Krucker, T., Smith, A.L., Lamp, T.C., Nelson, G.A., Obenaus. A. (2007). Effects of lipopolysaccharide on ^{56}Fe-particle radiation-induced impairment of synaptic plasticity in the mouse hippocampus. Radiation Research 168: 462-470.

Vlkolinsky, R., Titova, E., Krucker, T., Chi, B. B., Staufenbiel, M., Nelson, G. A., Obenaus, A.(2010). Exposure to $^{(56)}$Fe-particle radiation accelerates electrophysiological alterations in the hippocampus of APP23 transgenic mice. Radiat Res. 173: 342-52.

Wang, Y., Iqbal, J., Liu, Y., Su, R., Lu, S., Peng, G., Zhang, Y., Qing, H., Deng, Y. (2015). Effects of simulated microgravity on the expression of presynaptic proteins distorting the GABA/glutamate equilibrium--A proteomics approach. Proteomics.15(22): 3883-91.

Wang, Y., Javed, I., Liu, Y., Lu, S., Peng, G., Zhang, Y., Qing, H., Deng, Y. (2016). Effect of Prolonged Simulated Microgravity on Metabolic Proteins in Rat Hippocampus: Steps toward Safe Space Travel. J Proteome Res. 15(1):29-37.

Washburn, D.A., Rumbaugh, D.M., Richardson, W.K., Gulledge, J.P., Shlyk, G.G., Vasilieva, O.N. (2000). PTS performance by flight- and control-group macaques. J Gravit Physiol. 7(1): S89-93.

Wassersug, R., Izumi-Kurotani, A. (1993). The behavioral reactions of a snake and a turtle to abrupt decreases in gravity. Zoolog Sci. 10: 505-9.

Yamada, M., Kasagi, F., Mimori, Y., Miyachi, T., Ohshita, T., and Sasaki, H. (2009). Incidence of dementia among atomic-bomb survivors – Radiation Effects Research Foundation Adult Health Study. J Neurological Sciences 281: 11 – 14.

Zeredo, J.L., Toda, K., Matsuura, M., Kumei, Y. (2012). Behavioral responses to partial-gravity conditions in rats. Neurosci Lett. 529:108-11.

GOAL 7 References:

Alberts, J.R., Ronca, A. (2005). Development as adaptation: a paradigm for gravitational sand space biology. Adv. Space Biol. Med. 10: 175 – 207.

Albrecht-Buehler G. (1991). Possible mechanisms of indirect gravity sensing by cells. ASGSB Bull. 4(2):25-34.

Alwood, J.S., Ronca, A.E., Mains, R.C., Shelhamer, M.J., Smith, D.J., Goodwin, T.J. (2017). From the bench to exploration medicine: NASA life sciences translational research for human exploration and habitation missions. Microgravity (2017) 3:5. doi:10.1038/s41526-016-0002-8.

Anken, R.H. (2003). Neurophysiology of developing fish at altered gravity: Background - facts - perspectives. Adv. Space Biol. Med. 9: 173 - 200.

Blaber, E., Sato, K., Almeida, E.A.(2014). Stem cell health and tissue regeneration in microgravity. Stem Cells 23 Suppl 1: 73 - 78.

Boyko, A., Kovalchuk, I. (2011). Genome instability and epigenetic modification - heritable responses to environmental stress? Curr. Opinion in Plant Biol. 14: 260 - 266.

Hateley, S., Hosamani, R., Bhardwaj, S.R., Pachter, L., Bhattacharya, S. (2016). Transctiptomic response of Drosophila melanogaster pupae developed in hypergravity. Genomics 108(3-4):158-167. PMID:27621057.

Hemmersbach, R., Voormanns, R., Briegleb, W., Rieder, N., Häder, D.P. (1996). Influence of accelerations on the spatial orientation of *Loxodes* and *Paramecium.* J Biotechnology 47: 271-278.

Horn, E.R., Gabriel, M., (2015). Gender-related sensitivity of development and growth to real microgravity in Xenopus laevis. J Exp Zool A Ecol Genet Physiol 321: 1 - 12.

Hosamani, R., Leib, R., Bhardwaj, S.R., Adams, C.M., Bhattacharya, S. (2016). Elucidating the "Gravome": Qunatitative proteomic profilling of the response to chronic hypergravity in Drosophila. J Proteome Res. 15(12): 4165-4175. PMID: 27648494.

Ibsch, M., Anken, R.H., Rahmann, H. (2000). Weightlessness during spaceflight results in enhanced synapse formation in a fish brain vestibular nucleus. Neurosci Lett 296: 13 - 16.

Ikenaga, M., Yoshikawa, I., Kojo, M., Ayaki, T., Ryo, H., Ishizaki, K., Kato, T., Yamamoto, H., Hara, R. (1997). Mutations induced in Drosophila during space flight. Biol Sci Space. 11(4):346-350. PMID:11541768.

Jablonca, E. (2009). Transgenerational epigenetic inheritance: Prevalence, mechanisms, and implications for the study of heredity and evolution. Quarterly Rev Biol. 84: 131-176.

Kiss, J., Katembe, W.J., Edelmann, R.E. (1998). Gravitropism and development of wild-type and starch-deficient mutants of *Arabidopsis* during spaceflight Physiologia Plantarum 102: 493–502.

Klosin, A., Casas, E., Hidalgo-Carcedo, C., Vavouri, T., Lehner, B. (2017). Transgenerational transmission of environmental information in C. elegans. Science 356: 320-323.

Krasnov, I.B. (1994). Gravitational neuromorphology. Adv. Space Biol. Med. 4:85 - 110.

Leandro, L.J., Szewczyk, N.J., Benguría, A., Herranz, R., Laván, D., Medina, F.J., Gasset G., Loon, J.V., Conley, C.A., Marco, R. (2007). Comparative analysis of Drosophila melanogaster and Caenorhabditis elegans gene expression experiments in the European Soyuz flights to the International Space Station. Adv Space Res.40:506-512.

Ma, L., Ma, J., Xu, K. (2015). Effect of spaceflight on circadian rhythm, lifespan and gene expression of Drosophila melanogaster. PLoS One 10: e0121600.

Marthy, H. J. (2009) Editor. Developmenal biology research in space. Adv. Space biol. Med. Vol. 9. Elsevier, Amsterdam

Murata, Y., Yasuda, T., Watanabe-Asaka, T., Oda, S., Mantoku, A., Takeyama, K., Chatani, M., Kudo, A., Uchida, A., Uchida, S., Suzuki, H., Tanigaki, F., Shirakawa, M., Fujisawa, K., Hamamoto, Y., Terai, S., Mitani, H. (2015). Histological and transcriptomic analysis of adult Japanese Medaka sampled onboard the International Space Station. PLOS One 10(10) e0138799.

Mashinsky, A., Ivanova, I., Derendyaeva, T., Nechitailo, G., Salisbury, F. (1994). From seed - to - seed experiment with wheat plants under space-flight conditions. Adv Space Res.14(11):13-9.

Nelson, G.A. (2016) Space radiation and human exposure, a primer. Radiat. Res. 185: 349 - 358.

National Research Council (2011). Recapturing a future for space exploration. Life and physical sciences research for a new era. National Research Council. National Academies of Science. National Academies Press, Washington, DC.

Oczypok, E.A., Etheridge, T., Freeman, J., Stodieck, L., Johnsen, R., Baillie, D., Szewczyk, N.J. (2012). Remote automated multi-generational growth and observation of an animal in low Earth orbit. J R Soc Interface. 9:596-9.

Ogneva, I.V., Belyakin, S.N., Sarantseva, S.V. (2016). The development Of Drosophila melanogaster under different duration space flight and subsequent adaptation to Earth gravity. PLOS One PMID: 27861601.

Reitz, G., Bucker, H., Facius, R., Horneck, G., Graul, E.H., Berger, H., Ruther, W., Heinrich, W., Beaujean, R., Enge, W., Alpatov, A.M., Ushakov, I.A., Zachvatkin, Y.A., Mesland, D.A. (1989). Influence of cosmic radiation and/or microgravity on development of Carausius morosus. Adv. Space Res. 9(10):161-73. PMID: 11537289

Ronca, A.E., Fritzch, B., Bruce, L.L., Alberts, J.R. (2008). Orbital spaceflight during pregnancy shapes function of mammalian vestibular system. Behav Neurosci.122: 224 - 232.

Tash, J., Bracho, G.E. (1999). Microgravity alters protein phosphorylation changes during initiation of sea urchin sperm motility. *FASEB J*. 13: S43-S54

Wade, C. (2005) Response across the gravity continuum: hypergravity to microgravity. Adv. Space Biol. Med. 10: 225 - 245.

Warren, P., Golden, A., Hanover, J., Love, D., Shephard, F., Szewczyk, N.J. (2013). Evaluation of the Fluids Mixing Enclosure System for Life Science Experiments during a commercial *Caenorhabditis elegans* spaceflight experiment. Adv Space Res. 51(:2241-2250.

Wolgemuth, D.J., Herrada, G., Kiss, S., Cannon, T., Forsstrom, C., Pranger, L.A., Weismann, W.P., Pearce, L., Whalon, B., Phillips, C.R. (1997). Vertebrate development in the environment of space: models, mechanisms, and use of the Medaka. Gravit Space Biol Bull. 10:97-109.

Xu, D., Gao, Y., Huang, L., Sun, Y., (2014). Changes in miRNA expression profile of a space-flown *Caenorhabditis elegans* during Shenzhou-8 mission. Life Sci Space Res 1: 44-52.

GOAL 8 References:

Arena, C., De Micco, V., Macaeva, E., Quintens, R. (2014). Space radiation effects on plant and mammalian cells. Acta Astronautica 104:419-431.

Benton, E. Space Radiation Passive Dosimetry. (2012). The Health Risks of Extraterrestrial Environments (THREE), https://three.jsc.nasa.gov.

Budinger, T.F., Lyman, J.T., Tobias, C.A. (1972). Visual perception of accelerated nitrogen nuclei interacting with the human retina. Nature 239:209-211.

Cardis, E., Gilbert, E.S., Carpenter, L., Howe, G., Kato, I., Armstrong, B.K., Beral, V., Cowper, G., Douglas, A., Fix, J., Fry, S.A., Kaldor, J., Lave, C., Salmon, L. Smith, P.G., Voelz, G.L., Wiggs, D.L. (1995). Effects of Low Doses and Dose-Rates of External Ionizing Radiation: Cancer Mortality among Nuclear Industry Workers in Three Countries. Radiat Res 142:117-132.

Cardis, E., Vrijheid, M., Blettner, M., Gilbert, E., Hakama, M., Hill, C., Howe, G., Kaldor, J., Muirhead, C.R., Schubauer-Berigan, M., Yoshimura, T., Bermann, F., Cowper, G., Fix, J., Hacker, C., Heinmiller, B., Marshall, M., Thierry-Chef, I., Utterback, D., Ahn, Y.O., Amoros, E., Ashmore, P., Auvinen, A., Bae, J.M., Bernar, J., Biau, A., Combalot, E., Deboodt, P., Diez-Sacristan, A., Eklof, M., Engels, H., Engholm, G., Gullis, G., Habib, R.R., Holan, K., Hyvonen, H., Kerekes, A., Kurtinaitis, J., Malker, H., Martuzzi, M., Mastauskas, A., Monnet, A., Moser, M., Pearce, M.S., Richardson, D.B., Rodriguez-Artalejo, F., Rogel, A., Trady, H., Telle-Lamberton, M., Turai, I., Usel, M., Veress, K. (2007). The 15-country collaborative study of cancer risks among radiation workers in the nuclear industry: estimates of radiation-related cancer risks. Radiat Res 167:396-416.

Copeland, K. (2013). Occupational Exposure to Ionizing Radiation for Crews of Suborbital Spacecraft: Questions & Answers DOT/FAA/AM-13/23 Office of Aerospace Medicine Washington, DC.

Cucinotta, F.A., Manuel, F.K., Jones, J., Iszard, G., Murrey, J., Djojonegro, B., Wear, M. (2001). Space Radiation and Cataracts in Astronauts. Radiat Res 156: 460-466.

Cucinotta, F.A., Durante, M. (2006). Cancer risk from exposure to galactic cosmic rays: implications for space exploration by human beings. Lancet Oncol 7: 431-435.

Cucinotta, F.A., Kim, M.Y., and Chappel, L.J. (2013). Space radiation cancer risk projections and uncertainties. NASA/TP-2013-217375.

De Micco, V., Arena, C., Pignalosa, D., Durante, M., (2011). Effects of sparsely and densely ionizing radiation on plants. Radiat Environ Biophys 50:1-19.

Donà, M., Scheid, O.M. (2015).DNA damage repair in the context of plant chromatin. Plant Physiol. 168: 1206-1218.

Durante, M. (2012). Space radiobiology on the moon. Planetary and Space Science 74:72-77, 2012.

Fazio, G.G., Jelley, J.V., Charman, W.N. (1970). Generation of Cherenkov light flashes by cosmic radiation within the eyes of the Apollo astronauts Nature 228:260–64.

George, K., Durante, M., Wu, H., Willingham, V., Badhwar, G., Cucinotta, F.A. (2001). Chromosome Aberrations in Blood Lymphocytes of Astronauts after Space Flight. Radiat Res 156:731-738.

Hassler, D.M., Zeitlin, C., Wimmer-Schweingruber, R.F., Ehresmann, B., Rafkin, S., Eigenbrode, J. L., Brinza, D.E., Weigle, G., Bottcher, S., Bohm, E., Burmeister, S., Guo, J., Kohler, J., Martin, C., Reitz, G., Cucinotta, F.A., Kim, M.H., Grinspoon, D., Bullock, M.A., Posner, A., Gomez-Elvira, J., Vasavada, A., Grotzinger, J.P., MSL Science Team. (2013). Mars' surface radiation environment measured with the Mars Science Laboratory's Curiosity Rover. Science 343: 1244797.

Huff, J., Carnell, L., Blattnig, S., Chappell, L., Kerry, G., Lumpkins, S., Simonsen, L., Slaba, T., Werneth, C. (2016). Evidence Report: Risk of Radiation Carcinogenesis. Human Research Program ,Space Radiation Element, Johnson Space Center.

Jurist J. Human factors in commercial suborbital flight: Radiation exposure and weightlessness. (2005). http://www.thespacereview.com/article/464/1.

Köhler, J., Ehresmann, B., Zeitlin, C., Wimmer-Schweingruber, R.F., Hassler, D.H., Reitz, G., Brinza, D.E., Appel, J., Bottcher, S., Bohm, E., Burmeister, S., Guo, J., Lohf, H., Martin, C., Posner, A., Rafkin, S. (2015). Measurements of the neutron spectrum in transit to Mars on the Mars Science Laboratory. Life Sci in Space Res 5:6-12.

Möller, T., (2013). Measurement of the Radiation Exposure for High Altitude Flights in the Polar Region, Christian-Albrechts-Universität, Dissertation.

Moreno-Vilanueva, M., Wong, M., Lu, T., Zhang, Y., Wu, H. (2017). Interplay of space radiation and microgravity in DNA damage and DNA damage response. npj Microgravity 3(14) (doi: 10.1038/s41526-017-0019-7).

National Council on Radiation Protection and Measurements NCRP (2000). Recommendations of Dose Limits for Low Earth Orbit. NCRP Report 132, Bethesda MD.

Norbury, J.W. (1991). Transport Methods and Interactions for Space Radiations. NASA RP-1257.

Ohnishi, T., Ohnishi, K., Takahashi, A., Tangiguchi, Y., Sato, M., Nakano, T., Nagaoka, S. (2002). Detection of DNA damage induced by space radiation in Mir and space shuttle. J Radiat Res 43 Suppl:S133-6.

Pinsky. L. S., Osborne, W. Z., Bailey, J .V., Benson, R. E., Thomson, L. F. (1974). Light flashes observed by astronauts on Apollo 11 through Apollo 17 Science 183 957–9.

Pinsky, L. S., Osborne, W. Z., Hoffman, R. A., Bailey, J. V. (1975). Light flashes observed by astronauts on Skylab 4 Science 188 928–30.

Reitz, G., Berger, T., Matthiae, D. (2012). Radiation exposure in the moon environment. Planetary and Space Science 74:78-82, 2012.

Slaba, T.C., Mertens, C.J., Blattnig, S.R. (2013). Radiation Shielding Optimization on Mars. NASA TP-2013-217983.

Wilson, J.W., Townsend, L.W., Schimmerling, W., Khandelwal, G.S., Khan, F., Nealy, J.E., Cucinotta, F.A. Simonsen, L.C., Shinn, J.L., Norbury, J.W. (1993).Transport Methods nd Interactions for Space Radiations. In Swenberg, C.E., Horneck, G., Stassinopoulos, E.G. (eds) Biological Effects and Physics of Solar and Galatic Cosmic Radiation. NATO ASI Series, vol243B, 187-786.

Yang, T.C., George, K., Johnson, A.S., Durante, M., Fedorenko, B.S. (1997). Biodosimetry Results from Space Flight Mir-18. Radiat Res 148:17-23.

Yatagai, F., Honma, M., Takahashi, A., Omori, K., Suzuki, H., Shimazu, T., Seki, M., Hashizume, T., Ukai, A., Sugasawa, K., Abe, T., Dohmae, N., Enomoto, S., Ohnishi, T., Gordon , A., Ishioka, N. (2011). Frozen human cells can record radiation damage accumulated during space flight: mutation induction and radioadaptation. Radiat Environ Biophys 50:125-34.

Zhao, Y., Johnson, R. C., Baillie, D., Rose, A. (2005). Worms in space? A model biological dosimeter. Gravitational and space biology bulletin, 18(2):11-16.

Zeitlin, C., Hassler, D.M., Cucinotta, F.A., Ehresman, B., Wimmer-Schweingruber, R.F., Brinza, D.E., Kang, S., Weigle, G., Bottcher, S., Bohm, E., Burmeister, S., Guo, J., Kohler, J., Martin, C., Posner, A., Rafkin, S., and Reitz, G. (2013). Measurements of Energetic Particle Radiation in Transit to Mars on the Mars Science Laboratory. Science 340: 1080-1084.

Goal 9 References

Kim, J., Hammer, B., Harjani, R. (2012). A 5-399 MHz CMOS transceiver for multi-nuclear MMR spectroscopy. DOI: 10.1109/CICC.2012.6330645
https://pdfs.semanticscholar.org/11ab/73f01c2763bbc88729cb19712306e6b567c4.pdf

Liao, H., Kadjo, A. F., Dasgupta, P. K. (2015). Concurrent high-sensitivity conductometric detection of volatile weak acids in a suppressed anion chromatography system. Anal. Chem 87, 8342-8346 doi:10.1021/acs.analchem.5b01523