Excerpt from a NASA Developmental Biology Review Panel Report

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An external committee was formed with the purpose of providing advice to the Life Sciences Division, Office of Life and Microgravity Sciences and Applications of NASA, that would be instructive for planning for successful developmental biology studies on future NASA missions. The Panel represented a broad range of expertise related to neonatal development, including maternal and neonatal physiology and behavior, animal handling and husbandry, nutrition, cage design, and veterinary care. Members included individuals responsible for the conduct of research on mission shuttle flights, the development of scientific experiments flown on mission flights, and experts with no first-hand experience with NASA programs. Information for the development of this report was gained through background material provided on the Neurolab SpaceLab Mission, documents concerning Animal Care and Use, research articles on developmental biology in spaceflight, and from a fact-finding meeting held at NASA Ames Research Center (ARC),
August 10-12, 1998.

Developmental Biology Review Panel Members:

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**** The following is an excerpt from the above described NASA Developmental Biology Review Panel Report. This excerpt contains the Introduction and Science/Developmental Biology section, only, of that report.**

I. INTRODUCTION

NASA was established to provide for research into the problems of flight within and outside the Earth's atmosphere, and the agency has directed efforts to develop both the scientific and technological knowledge needed to ensure a permanent human presence in space. Since 1981, Space Shuttle missions have been launched to carry out the research essential for achieving this effort as well as for gaining new knowledge from space that contributes to the health, well being, and economic benefit of people on earth. Toward this goal, the Neurolab SpaceLab Mission was flown in April 1998. Studies on this mission explored the role of space flight and particularly the role of microgravity on the development, function, and behavior of the nervous system. Results from this mission provide a foundation needed to build future space flight research efforts in neuroscience and behavior.

Understanding the effects of space flight on the normal development of the nervous system was identified as one of the prime areas of research on the Neurolab SpaceLab Mission. This scientific area has also been identified as one of the highest priorities for research by the Committee on Space Biology and Medicine (CSBM) of the National Research Council[1]. Indeed, understanding the complete life cycle of vertebrates in space and the development of the brain, especially the gravity-sensing systems, is critical for ensuring the feasibility of long-term manned space habitation and exploration.

While extensive data were obtained about the effects of gravity on the development of the nervous system, and these results are being analyzed, problems arose with the survivability of young rats launched at nine days of age during the 16 day Neurolab SpaceLab Mission. This was unexpected since in a previous flight designed to test the health and survival of young rodents in flight, 19 of 20 eight-day-old rat neonates survived. As a result, NASA requested that a special panel be established with the charge of analyzing possible reasons for the poor survival of these young rats on the Neurolab SpaceLab Mission and developing recommendations to enhance success of future developmental biology research with rodents in space. Although the focus of the report is on studies of developmental biology and neonates, the recommendations have general applicability to animal studies in space.

SCIENCE/DEVELOPMENTAL BIOLOGY ISSUES

This section focuses on developmental neurobiology issues rather than the whole scope of developmental questions that are pertinent to space biology, since the expertise of this scientific Panel resides in neurobiology and development.

A. Importance of studying developmental biology and fertilization in space

Given the exciting advances and opportunities in the exploration of space, it is clear that we will continue in this endeavor. In the near future we will undoubtedly travel long distances and live for extended periods in spacecraft or on space stations. Thus, increasing numbers of people will spend increasing amounts of time under conditions for which the evolution of our species has not prepared us. While our experience with space life is limited, we are already aware that humans frequently experience major health problems associated with bone, muscle, and nervous system dysfunction during and after prolonged space flight. These medical problems must be dealt with to facilitate space exploration. Thanks to the cooperation of the international community of astronauts, clinical data has been gathered already that has begun to define the range and scope of the problems.

The human data have been helpful, but the ability to make meaningful progress in solving many space health problems will depend on extensive use of experimental animals. These studies will require a reliable supply of animals across the entire life span. While one way to ensure availability is to continually deliver Earth-raised animals to space labs, the expense of these deliveries would be very high. Further, there may be advantages to using animals that are pre-adapted to the weightless environment. Also, if research is to be conducted during longer, perhaps interplanetary trips, there will be no opportunity to restock the animal colonies. Therefore, animals for use in space research will have to be bred in space. As a consequence, we need to determine whether organisms can undergo fertilization and normal development in microgravity. In addition to this, in the future we need to learn whether human pregnant females and human neonates can fly in space, and live in microgravity on space stations during critical periods of development. Finally, there are some developmental processes that can be investigated uniquely in microgravity rather than on Earth, and it is important to the progress of science to pursue this work in space.

From this overview, it is apparent that there are both short- and long-term goals for developmental biology that should be pursued in space. The short-term goals are: (1) investigate the health-related problems encountered by astronauts in space, including bone, muscle, and nervous system dysfunctions during and after prolonged space flight. These health problems may be addressed in part by studying the development of the affected systems, including the vestibular system, muscle, and spinal cord development. (2) Determine whether fertilization and reproduction can occur normally in space. (3) Study developmental processes that can be investigated uniquely in microgravity, including studies on the development of the vestibular system. postural control and autonomic function.

The long-term goals for developmental biology studies conducted in space include: (1) Determine whether colonies of scientific animals can be bred in space laboratories. (2) Determine whether human pregnant females and human neonates can fly in space, and live in microgravity on space stations during critical periods of development.

B. Advantages/disadvantages of different vertebrate model systems

A major question addressed by the Panel is whether NASA should perform research on vertebrates and invertebrates, on mammals only, or include non-mammals. Since no critical effects of microgravity have been observed in model invertebrates[2,3], the highest priority should be given to test vertebrate models, such as fish, birds and small mammals, including mice and rats. Most of the research performed on Neurolab was conducted on rats. The advantages of the rat animal model include relatively small size and a large database on rat biology. However, from NIH-R3 and Neurolab, it is apparent that there are serious logistical problems in maintaining maternal nutrition of rat neonates under conditions of microgravity. One way to address this problem is to design rat cages that facilitate maternal care in space, which NASA is doing, and which is discussed further in the Veterinary and Hardware sections below.

For future space studies, it may be advantageous to use genetically altered mouse lines. This would allow researchers to capitalize on existing genotypes as well as to develop new model systems that would enhance our understanding of space biology and medicine. For example, the development of mouse "knockouts" for various elements of the otolith/vestibular system could lead to new insights into the effects of microgravity by studying this system in land-based experiments and comparing the results to those from mice developing in space. Fortunately, much of what we have learned about rats in space is likely to transfer to mice. Mice are known to be excellent mothers and communal breeders. Three female mice will occupy the space of one rat, and consume the same amount of food. Moreover, three related mothers in a nest area may increase the likelihood of neonate survival. If mice are flown in space, more Earth-based research on the mouse maternal system would need to be performed. It is already clear that there are differences between maternal behavior in mice and rats. For example, anosmia, loss of the sense of smell, disrupts maternal responsiveness in mice, but not in rats[4]. Thus, if litters of mice were flown in space, it would be important to provide a cage environment that allows the dam to smell her offspring.

A third approach is to consider for space studies mammals that develop within a mother's pouch, such as the opossum. A fourth possibility is to choose a more precocial species that undergoes most of its development during fetal stages, and thus avoids a neonatal period highly dependent on maternal care. A precocial mammalian candidate is the guinea pig. Another precocial species is the chicken, since the chicken can stand and feed itself within hours of birth[5]. There are other advantages to selecting the chicken as a developmental model. One is the ease of flying eggs versus neonatal rodents. In addition, there is a wealth of information available on the normal structure, function and development of the chick embryo. Already, chick embryo eggs have been flown in space, with varying degrees of success[6]. During a 9-day flight, two-day and nine day old chick embryo eggs were transported. While the E2 embryos died, the E9 embryos survived. Some of the E9 embryos were allowed to hatch on Earth, and behavioral tests performed 30-60 minutes after hatching indicated no abnormalities in the function of the vestibular system. This is obviously just a preliminary study, and a more in depth analysis of embryos at different stages is needed to evaluate the advantages of using chick embryos, or quail embryos, for space studies. A final point, if chickens can be raised in space, they may provide a practical source of food during long-term space habitation.

C. Recommendations for specific research opportunities in space

Since experiments conducted in space are complicated and expensive, NASA is judicious about identifying the most important studies to pursue in its space biology program, and draws on scientific advice from the CSBM of the National Research Council. Recently, the CSBM updated and reassessed its 1987 strategic plan (and 1991 follow-up assessment) on space biology research. The new strategic plan, "A Strategy for Research in Space Biology and Medicine in the New Century" [1], contains current scientific knowledge acquired by the CSBM from its members and also from its workshops composed of participants from diverse fields pertinent to space biology. In this new strategic plan, several specific topics of high priority to pursue in space were identified, including: 1) Grow organisms through two complete life cycles in space to determine whether microgravity affects normal developmental events. 2) Analyze the development of the gravity-sensing systems, including the vestibular system and other systems that interact with it. 3) Investigate the role of microgravity on the development and maintenance of neural space maps. 4) Characterize neuroplasticity, focusing on the plasticity observed in the vestibular system. 5) Study the development of the vestibuloautonomic pathway and its influence on cardiovascular output and pulmonary function. 6) Study muscle cell genesis and differentiation, and the development of the neuromuscular junction and spinal cord. 6) Study muscle cell genesis and differentiation, and the development of the neuromuscular junction and spinal cord. The report provides the strategy and rationale for prioritizing the research and strongly emphasizes the importance of ground-based programs.

The Panel concurs with the report of the CSBM that research opportunities in space should be limited due to the expense and difficulties in performing them. Since an important aspect of NASA's space program is to continue flying manned space missions, there is an obvious need for greater understanding of both the short- and long-term consequences of microgravity that would help to reduce the problems of "space adaptation". Thus, the most appropriate research problems and those that are critically related to the health of astronauts should be implemented in space. However, as recommended in the CSBM report, extensive work should be done on Earth before a research problem is considered ready for space. For example, some aspects of the problems of muscle atrophy, calcium loss in bones, and muscle disuse are easier and less expensive to investigate experimentally on Earth. Similarly, the problem of space sickness can be duplicated on Earth, and possible pharmacological interventions can be tested readily on Earth. Only after such Earth-based research has advanced should space studies be considered. Thus, the Panel recommends that NASA support more ground-based research in the life sciences, and adapt a conservative approach towards sending projects into space.

Major systems focused on sensing and responding to gravity are the vestibular system, the postural control system, and the autonomic nervous system. The vestibular system functions through pathways connecting the gravity-sensing receptors in the inner ear with neurons located in discrete regions throughout the brain. These brain centers in turn influence the muscles controlling eye movements, posture, and balance[6]. The postural control system involves tendon and joint receptors and associated spinal cord circuits, motoneurons and muscles. The autonomic nervous system provides homeostatic regulation of blood pressure and body fluids. These are critical areas of focus for space research. Specific issues to be addressed have been detailed in the CSBM report and this subgroup agrees with their importance.

The need to further explore nervous system function in space was emphasized by NASA in its support of the Neurolab space mission, which was focused on understanding the affects of microgravity on nervous system development. Specific Neurolab projects included studying the effects of microgravity on the neural and thyroid regulation of myosin expression in developing skeletal muscle; the development of neurons and synapses in the hippocampus; cerebellar cell proliferation, migration and differentiation; glutamate receptors and synapse plasticity in the vestibular nuclei and maculae; the development of the aortic arch baroreflex and its control of the cardiovascular system; motor behavior in the developing spinal cord. Altogether, the projects flown in the 1998 Neurolab mission are consistent with projects proposed for space studies in the CSBM report[1].

Finally, the Panel believes that certain scientific goals should be given priority as noted in the list below. Please note that the Panel recognizes that there is a wide range of developmental questions pertinent to space biology[1]. Given the expertise of the present scientific Panel, the focus is primarily on developmental neurobiology and behavior. The Veterinary Issues section includes recommendations that may be useful to enhance the survivability of neonates in space.

II1. Investigate the molecular and cellular basis of mechanisms underlying the vestibular compensation that astronauts undergo entering and returning from space and determine whether the underlying mechanisms in the two cases are shared, or how they differ.

II2. Study the development of the vestibular system and other systems involved with responses to gravity, including the proprioceptive, visual, autonomic nervous system, and also the multiple neural space maps in the brain. Much of this work involves studies performed on Earth to elucidate the underlying neural connections and fundamental processes, and should be performed in advance of experiments testing the effects of microgravity on these systems.

II3. Complete two-life cycles in space. In meeting the challenge of completing multiple life cycles in microgravity, there are several key milestones. The first is fertilization. All species have more or less elaborate mating rituals, and it is unclear how the lack of gravity will affect these. Tests are needed to determine whether natural mating will occur in the existing type of cage environment, or whether the cage design should be modified to encourage mating. Artificial insemination, and even in vitro fertilization (IVF), is now routinely performed in mammals. IVF is complex, and should be considered only as a last alternative. The payload advantage of artificial insemination is that on lift off from Earth, only a small number of females and several tubes of frozen sperm are required, which then can generate an expanded colony in space.

II4. Investigate the next developmental milestone, gastrulation and neurulation, including implantation in mammals. The death of E2 chicks in space[6] raises concerns about using animals that have large yolk sacs.

II5. Follow subsequent embryonic development up to and including birth (or hatching) in space.

II6. Examine suckling behavior in newborn mammals in microgravity, an area that clearly is of major importance in achieving survival of neonates.

II7. Evaluate effects of aging on the course and reversibility of adaptive or degenerative changes, especially in the nervous system. The data from John Glenn's recent space flight site is of interest in this regard.

II8. Evaluate the entire life history to determine whether males and females raised from the one-cell stage in space can propagate in space, and exhibit normal capacities. If structural and behavioral abnormalities exist, they should be characterized. It is important to determine if deficits are permanent, or if they can be reversed after a certain time by living under conditions of normal gravity.