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BEHAVIORAL BIOLOGY OF
MAMMALIAN REPRODUCTION AND DEVELOPMENT
FOR A SPACE STATION

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PART I

INTRODUCTION

The seventh shuttle flight brought the United States yet another step closer to establishing a permanent space station by the early '90s. Four of the five astronauts -- the largest crew yet -- were scientists as well as pilots, emphasizing the goal of working in space rather than simply getting there. The most important tasks of the mission involved launching two commercial satellites and using a remote manipulator arm to grasp another floating outside and stow it in the cargo bay. The crew also tended to 21 experiments, mostly commercial payload, which turned the shuttle into an orbiting factory. McDonnell Douglas, for instance, used electric fields to separate biological compounds with greater purity and in greater quantity than is possible on earth, and a Munich firm spent \$26 million on a module of eight experiments. Space industrialization may eventually pay for the space program, and perhaps even turn a profit.

But the flight was not only a technological and engineering triumph. For many who watched the first American female astronaut climb into space, it was also a triumph for American women, symbolizing their progress. As flight engineer and operator of the remote manipulator arm, Dr. Sally Ride was a vital member of the crew. For many scientists, however, Ride also symbolized the limits of our knowledge about how human life and other biological processes adapt to conditions of space.

Female reproductive physiology may be more vulnerable to damage from space conditions than is that of male mammals. At puberty, a female's gametes develop into eggs and move to her ovaries, where they are stored throughout her reproductive life. These are all the sex cells she will ever have and if damaged, the insult will remain and may thereafter affect her reproduction. In contrast, male gametes, the sperm cells, are normally replaced every few weeks, so in theory, there is less risk of permanent insult.

NASA has developed unevenly. The physical sciences and engineering have blossomed with the support and encouragement of numerous programs. The progress and contributions made by these disciplines are exemplary. Meanwhile, the life sciences have not enjoyed as much support and have made relatively little headway. Our astronauts represent this imbalance: Engineering sophistication can put them into an environment which may have harmful effects that are not known or are not understood -- because of biological naivete.

In fact, some facets of the space environment are already known to be stressful, teratogenic, and basically unfriendly. Although the database is meager, we have evidence that terrestrial organisms, including mammals, sustain physiological imbalances during and subsequent to extended exposure to null gravity. In particular, the weightless condition is associated with redistribution of body fluids, variation in electrolytes, altered calcium metabolism and breakdown of bone tissue, and changes in the size, tone, and strength of muscles.

Although there have been no major human disasters in space so far, our track record is probably the result of luck, excellent management, and short flights. However, as flights become longer and man penetrates space more deeply, our ignorance of how space effects fundamental life processes will increase the risk, and our luck may run out.

But there is more to the roles of life sciences in space than diagnosis or prevention of ailments. The life sciences are a dynamic and vital part of human culture. Within the sciences generally, the life sciences in particular are enjoying explosions of discovery and progress. From molecular genetics to immunology to the behavioral and neural sciences and to evolutionary biology, we are in the midst of waves of excitement and innovative activities. Much of the exploration that characterizes these endeavors is of interest not only to the scientists that conduct it, but to the public as well, for these are inquiries that tell us more about the nature of life on earth, giving us deeper glimpses into ourselves, our origins, and maybe even into our future. This is the stuff that culture is made of, and I believe that among the multiple roles of the life sciences in NASA is the role of contributing to these aspects of culture.

A LIFE SCIENCES RESEARCH PROGRAM

NASA's plans for a space station not only require an expanded life sciences research program but also makes one more feasible. The modest number of shuttle flights and their short duration place severe limitations on research. Many of the most pertinent and important questions to be asked by life scientists concern adaptation by organisms to space conditions. Two kinds of adaptation are important problems to pursue, and both will be discussed in the course of this report. One kind is somatic adaptation -- the adjustments made by an organism, within its lifetime, in response to local conditions. The study of somatic adaptations to space is currently recognized as a priority issue. Flights of one week and even one month are not sufficiently long to conduct thorough studies of somatic adaptation. A permanent facility will allow proper research to

be conducted and set the stage for the parametric studies required to deal with the implications of the inevitably longer and more frequent exposures that will accrue as space station activities expand.

The second kind of adaptation is transgenerational adaptation. In contrast to somatic adaptations, which occur during an individual's lifetime, the transgenerational kind involves continuous exposure across sequential life cycles of genetic descendents. Only a permanent facility can support such work. Transgenerational adaptation is important to study for different reasons than the somatic kind. Transgenerational effects are akin to evolutionary processes. It is conceivable that space may become a laboratory for studies of evolution of life on earth. In the space environment, the lack of earthly constraints may effectively "unleash" processes which are normally invisible to us and give us the opportunity to witness and to study the determinants of the creation and variation of our living world. For instance, some scientists discuss the concept of "cytological memory," a notion that the encoding and storage of information by sex cells may be affected by or include information pertinent to environmental forces such as gravity. Thus, phenotypic expression derived from sex cells formed on earth might still reflect the terrestrial influences. It could not be until the next generation, with individuals whose sex cells have no such "memories," that the full adaptational changes could be seen.

It is not necessary to speculate about altered genes or germ cells to justify the study of transgenerational effects. Even organisms that sustain longterm exposure to space may retain in their morphology, physiology, and behavior, the legacy of development on earth. Modern biology has alerted us to the power of adaptational potential in life systems, but we have yet to explore the limits of that potential. One of the inestimable opportunities offered by a space station is in this realm.

Another desirable prospect of space station research is that the "shirt sleeve" environment would offer researchers the first opportunity to incorporate into the conduct of space science some of the creative processes that characterize research as it is performed on earth. I refer to the ability of hands-on researchers to observe, deduce, and respond -- to alter the course of an experiment because a new finding or observation points them in an unexpected direction. Some of the studies discussed in this report have such qualities. To conduct such research properly, we would want experienced researchers to have the opportunity to react to the phenomena they see and to take their studies in unanticipated directions if they encountered unanticipated phenomena.

A space station offers the opportunity to ask a new

generation of questions concerning whether, to what extent, and in what manners gravity is an organizing influence on life processes. Some of these questions will be of both pure and applied interest. Studies of vestibular function and proprioception, the mechanisms that give us the "sense" of gravity and acceleration and tell us the position of our body and limbs, have been neglected on earth but deserve special attention in space. Such investigations are of interest in basic research because it has been difficult to understand the action of gravitational stimuli when they cannot be eliminated for experimental purposes. These investigations are also of interest because they apply to analysis of "human factors" and performance in space.

BEHAVIORAL BIOLOGY FOR A SPACE STATION

The areas of a Life Sciences Program in a space station that will be addressed in this report concern, specifically, questions of the behavioral biology of mammalian reproduction and development, using the Norway rat as the focus of experimentation. In a later section of this introduction, I will examine the choice of the rat as a research animal, but first I would like to describe some features of behavioral biology that support my belief that it is a discipline that NASA should incorporate into its life sciences program and, specifically, in plans for a space station.

Behavioral biology, as the term suggests, is concerned with the study of biological aspects of the behavior of organisms. These concerns cover an enormous range of interests and techniques. The unifying theme, however, is a dedication to integrative functions of the organisms which is, after all, much of what we call "behavior." Thus, behavioral biology is concerned with the result or outcome of the myriad chemical, neural, and anatomical processes that subserve life processes. Mechanism is a valuable concern and often a specific interest, but usually because it will clarify the basis of behavior. Endocrinology, for instance, is a relevant specialization because hormones affect behavior and are affected by behavior.

As an integrative specialization, behavioral biologists can enjoy a special role with respect to other, more molecular, specialists. My work on the upcoming Cosmos flight is a pertinent example. I am collaborating with a developmental neuroanatomist, Dr. J. Richard Keefe, who will be performing morphometric studies of structures in the visual system of rats that have been in space. I will test their visual function. Keefe may spot anatomical differences between the visual system of the flighted animals and animals in the control group. What will it mean? The interpretation of his finding may rest on the functional significance of the anatomical variation: A

difference has to make a difference to be a difference. Alternatively, anatomists in Keefe's situation are faced with the overwhelming task of having to choose to make a finite number of measures from an enormous set of possibilities. He may conclude that there are no differences. The functional tests might say otherwise. This discrepancy would flag the anatomist: Go back to the specimens, for there is probably a significant difference in there. Try some of the additional measures.

Behavioral biology is not only a useful device for unifying and integrating various reductionistic specializations, but it is also a discipline with special and useful tools of its own. These tools, which are both technical and analytic, have been developed and tested mostly over the past decade or two, as the discipline has come of age.

Dyadic interactions

One of the analytic tools of behavioral biology that I believe may be useful to space station applications comes from the analysis of special types of behavioral interactions. One of the distinct advances in these analyses has been the adoption of a perspective whereby patterns of interactions between individuals are treated as a system. Such interindividual interactions, involving two players, are called dyads and their behavioral patterns are called dyadic interactions. One useful tool of contemporary behavioral biology is the systematic analysis of complex, multi-leveled, dyadic interactions. Sexual reproduction by mammals is a good example of a dyadic interaction. It involves interactions on sensory, behavioral, neural, and endocrine levels.

The concept of dyadic interactions implies inter-individual controls: The behavior of one participant affects the behavior of the other. In the type of interactions referred to as "multi-leveled," the interactions involve different levels of organization. For instance, the behavior of Participant A can affect the physiology of Participant B. Then, physiological changes in B can result in alterations in the attributes of B which, in turn, can exert changes in the behavior and/or physiology of Participant A.

The "systems" that arise from dyadic interactions are based on the exertion of effects by each participant on the other. In order to analyze such systems and to diagnose breakdowns, we must use appropriate tools. Behavioral biology has developed such tools. One of the most common is a method of dissociating major, interactive elements in the dyads and inserting for one participant a substitute player whose behavior or physiology is known and, ideally, under the control of the experimenter. This, combined with observational techniques of sequential

patterns of interaction, can reveal whether the system is breaking down because of a failure in one or both participants and which links in the chain of interactive events have been affected.

To appreciate the importance of these ideas, consider the interpretive problems that arise when a pair of animals fails to mate. The range of possibilities is staggering. The male could be disrupted, the female could be disrupted, or they both could be. They may be willing and eager to mate, but a malfunction in endocrine function in one (or both) might prevent consummation. The male and female might be able and willing, but one of them lacks a critical cue or does not fully express a social signal that is needed to "turn-on" the other. Or, the cues are all there, but one of the participants has been perceptually impaired and does not accurately sense the presence of the signal. The list can go on. The point is that it is essential to watch carefully, to collect the important data as the interactions proceed, and to be able to arrange different combinations of tests to diagnose problems and then to restore the functional integrity to these delicate but vital dyadic systems. Behavioral biology provides the tools for these procedures.

The concept of dyadic interaction is directly applicable to both mammalian reproduction and development. Mating behavior is a classic case, involving the coordinate interactions of male and female. The developmental process in mammals also exemplifies dyadic activities; Here, the players are parent and offspring. We will draw upon this perspective throughout the forthcoming report.

Movement notation and analysis

An analytic and technical innovation of behavioral biology is the design and application of different systems of movement notation. A variety of systems, based on different principles of description, are available. They are applicable to the behavior of individuals and of groups. One of the virtues of these systems is that many are amenable to quantitative methods, putting meat on the bones of observation. Another virtue is that many of these systems provide perspectives that are helpful in revealing patterns of action that are otherwise hidden. These perspectives vary in applicability to different situations. Some describe behavior in relation to a space. Others describe behavior as actions of the body in relation to itself and focuses on repetitions of patterned movements within this framework. I predict that these systems of movement notation can make invaluable contributions to the quantitative analysis of behavior in null-gravity settings and that they should be incorporated into plans for biobehavioral studies in space. The application of these methods to group behavior is

also new and important. Here, too, we shall see applicability to space station investigations.

Behavioral ecology

Part of the heritage of modern behavioral biology is the discipline of ecology, particularly those aspects of environmental analyses that pertain to the adaptive organization of animals to the habitats and niches in which they evolved. This perspective fosters a sensitivity to the inextricable connections between organismic function and environment. Behavioral biology would bring to the design of space station facilities and research valuable knowledge and a useful approach that could make the difference between success and utter failure in the establishment of functioning colonies of animals in an alien environment. In this report, in fact, I make frequent use of principles of behavioral ecology in the design and justification of various habitats that I think may be crucial for space station research with animals.

WHY REPRODUCTION AND DEVELOPMENT?

The choice of mammalian reproduction and development as a premiere problem for space station research has attractions for both pure and applied scientific reasons. From the standpoint of basic human knowledge, it has been said that "organisms are life cycles that reproduce" and that evolutionary changes are shifts in those cycles, expressed through changes in development. In addition, developmental studies represent an opportunity to study fundamental organizational mechanisms. Thus, to investigate reproduction and development brings us directly into the major arenas of the life sciences, grappling with the rudiments of the dynamic organization of living systems. It is an introduction to some of the most challenging intellectual endeavors that human beings can undertake. To the extent that space biology represents, in part, a framework in which to study earth life in relation to other worlds of the universe from which it arose, reproduction and development represent the "final common path" of the process of creation and variation.

Among the more "applied" reasons for the choice of reproduction and development are some of the anticipated needs that will arise with the establishment of a permanent space station. Numerous users of a space station will need access to research animals. NASA has clearly anticipated these needs, as evidenced by their interest in RAHF, the Research Animal Holding Facility, to be tested on the upcoming Space Lab-3. Its applications will vary: Biomedical researchers in need of animals for in vivo tests; industrial specialists, perhaps from

pharmaceutical industries needing subjects; physiologists, anatomists, and geneticists needing subjects; and, if possible, space-adapted subjects. Thus, the study of reproduction and development in animals in a space station meets an anticipated need of other scientists that will use the facility. The issues of animal maintenance are complex. The type of approach that I will recommend should help clarify many of these issues.

Another aspect of applied significance of these problems relates to human presence in space. The existence of a permanent space station will result in more humans spending more time in space. As I have indicated, it is inevitable that we will have to conduct parametric studies of space adaptation -- and readaptation -- to protect the well-being of the people that live and work in a space station and return to earth. Research animals will undoubtedly be needed to support the biomedical studies of space effects that will be required for human welfare.

Early in this introduction, specific reference was made to Sally Ride, the first American female astronaut. Among her many symbolic roles is the grim reminder that space biology has virtually ignored specific investigations of female physiological responses to space conditions. To date, the total census of female mammals that have been in space include: two Soviet female cosmonauts, one American woman astronaut, five female rats on the Soviet's Cosmos-1129, and one Apollo 17 pocket mouse. All other mammals in space have been males! (See Appendix J) Studies of reproduction and development both represent a conscious effort to rectify this improper and potentially disastrous imbalance. In addition to differences in reproductive physiology, we must examine other possible gender-related differences in response and adaptation to space conditions.

WHY RATS?

"If someone were to give me the power to create an animal most useful for all types of studies on the problems concerned directly or indirectly with human welfare, I could not possibly improve on the Norway rat."

-- Dr. Curt Richter, Professor Emeritus
The Johns Hopkins University

Dr. Richter's judgement reflects the enormous contributions to scientific research that have been made by domesticated strains of the Norway rat (Rattus norvegicus). The present program of space station investigations of mammalian reproduction and development is designed to use the Norway rat as the subject of study. The long and illustrious history of the rat in laboratory research makes available an unparalleled

database on this mammal.

In addition to economy and expediency, there are substantive reasons to choose the Norway rat as our focal animal for space station research on reproduction and development. The rat is a prolific breeder. The female rat's estrous cycle is only four-five days long. That is, she is ready to mate nearly twice per week and is not highly seasonal in fecundity. Litters of six-12 pups are born after a gestation period of only three weeks. A post-partum estrous makes it possible for the mother rat to conceive and gestate a second litter while she is nursing the previous one. Weaning of the offspring occurs at about three-weeks after birth, which therefore makes it possible for the mother to gestate one litter and simultaneously nurse another through consecutive developmental cycles. The mother rat is a highly efficient breeding machine.

Offspring rapidly reach sexual maturity, usually within six or seven weeks of age. It is easy to understand how rat populations can increase explosively. The arithmetic of rat reproduction makes the organism a prime choice for efficient production of future generations in a space station.

The maturation of the Norway rat makes it similarly well-suited for the kinds of studies needed for a preliminary program of developmental studies. At birth, it is almost fetal in appearance: It is born without fur, with its eyes and ears sealed, and with only rudimentary locomotor abilities. In a later section of this report, I will review the maturational stages of the rat pup. One of the advantages of the rat's immaturity at birth is that many dramatic developmental processes occur after birth, within the sight and reach of the human experimenter. There also exist species of rodents that remain in utero through many of the developmental stages that the Norway rat displays postnatally. I will discuss some of these cases to illustrate further the usefulness of rodents for basic biobehavioral studies in space.

I believe that the Natural History of Rattus norvegicus provides further evidence of the special suitability of this species as a regular member of the research team on a space station. The Norway rat belongs to the biological Order of rodents, which includes other rats, mice, squirrels, and many other species. Rodents are the most successful of all living mammals in the sense that they outnumber all other mammalian groups combined. Their range of adaptations is wide, extending to nearly every inhabitable area on earth (see interview with Dr. B. G. Galef in Appendix A for more historical information).

The Norway rat belongs to the biological group renowned for its adaptability, and is one of the best and most versatile of all. Indeed, the modern, natural history of the rat is

integrally connected with human history, particularly those chapters involving travel, exploration, and adaptation. It is largely from this association that the rat also earned the poorer aspect of reputation -- as a dangerous pest.

Most species of animals, including many other rodents, have evolved adaptational systems to a particular, narrow niche (e.g., wet areas with overhead cover, insects to eat, and temperatures that range from 25 to 32 degrees). Dispersal is limited by the availability of suitable niches, and the range of habitat variation that they can tolerate is fairly narrow. In contrast, it is impossible to define the "species-typical" habitat of the Norway rat. The rat is acknowledged as one of the great "generalists" on the planet. Rats can (and have) adapted to virtually every habitat known to man, everywhere on earth. If we are to choose from all the available species on earth one other mammalian form to accompany us into space and set-up residence in a space station, the sum total of human history and rodent history would point to the Norway rat as one of the best bets. The rat has an excellent track record for successful adaptation to novel environments. Its dispersal and abundance are testimony to these abilities, including willingness to breed in a variety of habitats.

There are, of course, alternatives to the rat. As I mentioned, there are other rodent species whose reproductive strategies might make them good candidates for particular kinds of studies. Species such as mice (Mus musculus) are also excellent generalists and successfully invade novel niches. If size and weight are important factors, they might be more desirable. Their small size, extraordinarily rapid metabolic rate, and rapid movements, however, have made them less popular as research subjects for behavioral studies. Handling and observation are considered more difficult with mice.

In all, the Norway rat offers a remarkable constellation of traits. It has traveled far and wide with man thus far. It may be destiny that it comes with us to a space station.

SIGNIFICANCE OF RAT STUDIES IN SPACE

"Rats in Space." The image is both compelling and somewhat amusing. The significance of such an achievement, however, would be monumental. To establish and maintain a population of rats in space under conditions that would permit them to live and to breed successfully would open inroads into numerous avenues of important inquiries, some of which are of conventional biomedical significance, others are particularly relevant to industrial interests, and some have immediate potential for the advancement of empirical and theoretical

issues in the life sciences. In addition, I predict -- along with numerous other scientists who have shared their thoughts and intuitions on the prospect of a space station for research -- that such a facility would be a catalyst for new dimensions of current scientific investigation as well as the seed of studies of phenomena and questions that are presently not known or part of our scientific consciousness. To be more specific:

BIOMEDICAL SIGNIFICANCE

Sub-human organisms are a necessary part of basic biomedical research and testing, even when the sole purpose of the work concerns applications to humans. Modern biomedical knowledge is based upon continuity and sharing of cellular and physiological processes among mammals. Research and testing of drug effects, hormonal responses, disease reactions, etc. can be conducted with non-human organisms, before such humans are directly involved as subjects. Transfer of data across species always requires qualification to account for differences related to body size and shape, metabolic rates, and numerous adaptive specializations.

Similarities in biological function among mammals vastly exceed the differences, however, and often the differences are understood enough to make preliminary cross-species corrections. Standard laboratory species, such as rats and mice, form the foundation of the basic biomedical research that precedes human applications. The same process will apply to issues of space biomedicine.

Choice of species for biomedical studies. For some problems, of course, rats are not optimal non-human experimental animals. Certain important questions (e.g., cardiovascular adaptation or spinal compression) are better to study with monkeys since they share our needs related to vertical pumping and upright posture: four-legged species do not. Nevertheless, other fundamental questions, such as calcium metabolism, electrolyte balance, energetics of movement, spermatogenesis, muscular development, and numerous other biomedical questions can be studied initially with rats.

For many projects, rats may make better subjects than primates. Rats and mice have evolved to live in small spaces; their social and behavioral repertoires are commensurate with their niches. Primates, on the other hand, carry with them millions of years of evolutionary honing, aimed at adaptation to life in large, often semi-arboreal niches in which complex social networks maintain homeostasis. Coupled with their keen intelligence and explosive capacity for movement, they do not do as well in captivity. We are more likely to study an inadvertently stressed monkey than a stressed rat in a typical

laboratory investigation. Why invite such complications unless it is necessary?

The existence of a space station, manned periodically or permanently, will bring with it new opportunities for research and, simultaneously, create new needs for research. Utilization of a space station heralds long-term exposures to space conditions and therefore the need to study, in advance of serious problems, the processes of mammalian long-term adaptation to space and subsequent readaptation to terrestrial conditions.

Sally Ride's mission signifies many things, and among them is our present ignorance of how space conditions may affect female physiology. The prospect of a habitable space station means that women will be routinely exposed to space for extended periods. Currently, the complete census of female mammals that have been in space consists of : five Soviet-flown female rats (Cosmos-1129, in 1979), two Soviet cosmonaut women, one U.S. female astronaut, and one Apollo 17 pocket mouse.

Space biology and space biomedicine have, thus far, ignored basic studies of female responses to space. This has been foolish; plans for a space station make it possible and necessary to make-up this deficit. We must anticipate these and other needs and plan accordingly.

Standard procedures for preliminary biomedical investigations of adaptation and readaptation in laboratory animals should therefore be part of the fundamental concept of a space station.

SIGNIFICANCE FOR BASIC RESEARCH

In as sense, all of the life sciences is the study of the organization of life, from its biochemical to its behavioral mechanisms -- in the presence of gravitational forces. These mechanisms, and the complex organisms that they comprise, have evolved in the presence of gravity. We can begin to study the roles of gravity by "removing" it and studying terrestrial life forms in space. A weightless environment provides a laboratory in which we can learn about adaptations that are related to gravitational forces. These mechanisms are nearly invisible on earth. In space, they can be more easily revealed.

The present report is focused on unique contributions that behavioral biology can make to NASA, especially if the United States establishes its presence in space by introducing a manned space station. Behavioral biology has not been part of programmatic studies in space. This is a good time, and a space station would be a good place to initiate tradition.

Adaptation is one of the unifying themes for contemporary programs in space biology. Behavioral biology is the study of adaptation on different, integrative levels of behavior, from the activities of nerve cells and whole organisms to groups of organisms. We can use behavioral biology in a space station to investigate how the organism adapts to the space environment, particularly during and after long-term exposures. Shuttle-length flights are too short for the necessary studies. The second kind of study is multigeneration adaptation. For this, generations of animals must go through full reproductive-development cycles in space. A space station is necessary to accomplish such a feat.

I discuss a behavioral biologist's vision of how to create a space station breeding program for rats, one that is tailored to the animals and their well-being. The process of creating a reproductive colony of mammals in space and the availability of this research facility can revolutionize the study of adaptive mechanisms in biology.

A space station will be both a laboratory and a tool. In it, we can study adaptation -- somatic as well as transgenerational -- in the absence of constant terrestrial forces. There is no comparable situation on earth. A space station is a gem for behavioral biology.

APPLIED SIGNIFICANCE

The utilization of a space station for industrial and even profit-making purposes has just begun to be explored. Some of these industrial interests will require the availability of animals for testing. For instance, pharmaceutical research and manufacturing may go into a space station, where substances of extreme purity can be manufactured economically. Such biomedical products will require testing with animals. The availability of an on-board animal facility could determine whether the U.S. will establish or maintain leadership in different areas of industrial utilization of space. A space station plan should therefore include animal facilities and a related research program in order to establish such a pioneering resource.

ETHICAL ISSUES

The decision to use a space station for biological research with mammals raises the need to consider the ethical issues involved, primarily those related to animal welfare. This is best initiated before a space station is used and similarly, in

the case of this report, before we get into its content. For the scientific and historical reasons given above, I recommend the Norway rat as a candidate for basic biobehavioral research in a space station.

In making this recommendation, I believe it is also my responsibility to acknowledge the numerous ethical issues involved and to mention that I have made many efforts to include these considerations throughout the report because I believe that such concerns are valid and important for the welfare of the animals involved, the quality of the related scientific work, and the effect on society that is to support and take from the overall effort.

During the final stages of preparation of this report, I made initial contact and discussed the approach used in this paper with Dr. John McCardle, an official in the Humane Society of the United States (HSUS). Dr. McCardle, who is based in Washington D.C. and is familiar with many of NASA's current and past life sciences projects, is the HSUS's specialist in laboratory animal concerns. We discussed the perspectives and standards used by the HSUS to evaluate the animal welfare issues relevant to research. Two major issues emerged: First was the justification of a particular animal research project, whether the ideas are interesting, important, and new -- and that the feasible alternatives have been considered. The second class of issues concerned the quality and standards of maintenance of the animals.

The importance and attraction of space station research is, in part, that it is unique. There is literally no substitute or equivalent anywhere on earth. As I indicated above, at least in general terms, there are numerous needs for research animals that can be anticipated for a space station. I would expect that rigorous standards would be applied to the selection of the scientific studies that would be awarded a place in a space station.

Issues of animal maintenance and the well-being of the animals in a space station are primary concerns throughout this report. Indeed, the intellectual and scientific biases of the field of behavioral biology in general, I think, represent a formal acknowledgement of the importance of animal welfare in research aimed at valid, high-quality information. The intellectual heritage that spawned behavioral biology includes evolutionary/ecological concerns. These disciplines emphasize the intricate and vital relationship between organism and environment.

Thus, a major part of the program described in the present report is aimed at encouraging NASA to incorporate a vigorous life science program into its plans for a space station and to

do it in a manner that is exemplary, both on the basis of its scientific merits as well as its ethical concerns. Done in this manner, NASA can enjoy a role of leadership in another important domain of our society. The space station effort will also be enhanced in the sense that some of the life sciences work will be well-suited for appreciation by a larger portion of the general public. They will enjoy the view of life in space offered by an innovative, creative approach to behavioral biology, an approach that is best communicated visually, in terms that can be presented to a wide and appreciative audience.

In my initial telephone conversation with Dr. McCardle, I described briefly the approach I have taken in this report, and the perspective I have tried to embody in the design principles for animal studies. His initial appraisal was that he "couldn't agree more" with the concerns. I take this as a positive sign that the program described here is one that has the virtue of broad appeal and can be fashioned to satisfy the heterogeneous groups that it will represent.

SCOPE OF THIS REPORT

Ostensibly, this report is concerned with mammalian reproduction and development under conditions of null gravity, as revealed by rats in a space station. Embodied in these problems, however, are much larger issues: how to approach the initial uses of a space station to conduct a form of behavioral biology that is important scientifically and which is interesting and pleasing to the public, so they may be able to follow with curiosity and pleasure the initial explorations of earth life as it makes a home in the unknown environment of space.

Another message of the report is that behavioral biology is an important, integrative specialization that should be a regular part of NASA's space biology program. The time has come for an injection of special, vigorous support. Thus, development and reproduction are focal topics, but they are also a vehicle for the discussion of more general issues. There have been other essays and documents, such as the Fabricant report, that are concerned with life sciences research in a space station. None of these, to my knowledge, have focused on the level of the neural and behavioral sciences.

DEVELOPMENT OF THIS REPORT

A five-month contract supported the creation, design, and production of this document. Its development was thus rapid and

short. Among the most important tools used at every stage was the open exchange and feedback from other scientists. Mainly, I used telephone interviews. Despite the brevity and impersonal nature of such "meetings," these conversations were stimulating, constructive, and instructive. With the consent of each interviewee, most of the calls were tape recorded. About ten of the 25 interviews were transcribed and edited. These are included in the Appendix and are referred to throughout the text. The edited interviews are substantive parts of this report and provided detailed and unique sources of valuable information for NASA's space biology effort. Some of the interviewees were compensated with modest honoraria, to acknowledge their generous contributions of time and attention.

Most of the scientists that I interviewed are university and medical school researchers and leaders in their fields of specialization. I selected a roster of interviewees to span and sample a wide range of interests, approaches, backgrounds, and levels of seniority. They are a diverse group, ranging from developmental neurobiologists to dedicated behaviorists. They include at least one member of the National Academy of Sciences and this year's recipient of a national "Early Career" award for scientific achievement.

Despite their diversity and different degrees of familiarity with me, there were striking and consistent themes: One was that they were uniformly enthusiastic about the prospects for life sciences research in space. Each individual recognized a number of important lines of investigation that would be attainable with research opportunities in space.

Another common theme is that these researchers were uniformly shocked and dismayed when they learned how little NASA has investigated "integrative" levels of biological function, such as those manifested in animal behavior. Most of these interviewees are behavioral biologists. They were aware that NASA has been more dedicated to excellence and developments in the physical sciences and engineering than in the life sciences, but each still assumed that there had been more basic work with organismic biology of mammals than has been accomplished.

The message from these experiences: NASA has available a community of interested, willing, and enthusiastic scientists. They are creative, energetic, and excellent in their fields. They have visions for space biology and can see how they could both contribute to and benefit from research using space facilities and answering space-related questions. None of them were aware of the degree of retardation that the biobehavioral areas had endured. Each commented on the necessity for basic data in these areas, if only to support developments in other areas.

The present document is young. It was an adventure to write. The support and heroic efforts of co-workers, such as Ms. Sally Delgado, the artist, and Ms. Lori Sparzo, the editor, were particularly important components. The report is, nonetheless, still in a formative stage. More scientists could now be included in the interviews. More precise and specific research designs could be sought and explored.

We hope that this report will kindle and enhance interests in behavioral biology for a space station. Star Enterprises would be eager to participate further.

Jeffrey R. Alberts, Ph.D.

Research Director
Star Enterprises
Bloomington, Indiana

August, 1983

Dr. C. Robert Almlı (Washington University Medical School)

Developmental neurobiology; movement analysis in neonatal rats and humans; development of movement notation systems for assessment of brain dysfunction.

Dr. Ann Baker (Colorado State University)

Comparative rodent development; environmental influences on early development.

Dr. Peter C. Brunjes (University of Virginia)

Developmental neurobiology; anatomical measurements of rodent brain structures during development.

Dr. Christopher Cann (University of San Fransisco Medical School)

Physiology; Cosmos PI, conducting experiments on calcium metabolism and bone composition after null gravity.

Dr. Jill Fabricant (University of Texas Medical Center)

Geneticist and toxicologist; former NASA contractor, responsible for organizing numerous study groups on physiology applicable space station studies and author of the resultant "Fabricant Report".

Dr. B. G. Galef, Jr. (McMaster University)

Behavior and ecology of wild and domesticated Norway rats and other rodents.

Dr. Gayle Gross (University of North Carolina, Chapel Hill)

Developmental neurobiologist; high altitude physiology; vestibular development in embryos.

Dr. Myron A. Hofer (Albert Einstein School of Medicine)

Developmental psychobiology; measurement of autonomic nervous system activity and neural development in infant rats; mother-litter regulations of physiological balance.

Dr. J. Richard Keefe (BioSpace, Inc. and Case Western Reserve Medical School)

Cosmos PI; experience with developmental studies of amphibian, fish, and bird embryos in space; neuroanatomical development, particular interest in vestibular, visual, and olfactory systems; expert in space biology.

Dr. Seymour Levine (Stanford University Medical School)

Pituitary-adrenal measures of stress and coping; stress during pregnancy and its effects on mothers and offspring in rats and non-human primates; mother-offspring interactions.

Dr. John McCardle (Human Society of the United States)

Head official at HSUS, specializing in standards for laboratory research animals.

Dr. Martha McClintock (University of Chicago)

Rodent sexual behavior; group mating processes; olfactory influences on estrous synchrony and sexual behavior; behavioral endocrinology.

Dr. Emily Morey-Holton (NASA - Ames Research Center)

Space biology and physiology; calcium metabolism and bone structure; cellular physiology; general experience and broad perspectives on issues in space biology.

Dr. Ronald Oppenheim (Bowman-Grey School of Medicine)

Developmental neurobiology; spinal cord and brain stem systems; general expertise and wisdom in fields of neurobiology and behavioral embryology.

Dr. Jiro Oyama (NASA - Ames Research Center)

Gravitational physiology; extensive experience with hypergravity and reproduction and development in rodents; adaptation effects of hypergravity and readaptation to 1-g.

Dr. Eugene Sackett (University of Washington)

Primatologist; prenatal influences on primate development; mother-infant relations in primates; behavior coding systems and expert on quantitative, sequential analyses of behavioral interactions.

Dr. Timothy Schallert (University of Texas at Austin)

Movement and postural analysis in rodents; vestibular specialist; design of tests of sensory and motor impairment and recovery after neurological manipulations by drugs and surgery.

Dr. Christopher Schatte (NASA - Ames Research Center)

Project Scientist, Spacelab-4; vertebrate physiology.

Dr. Kenneth Souza (NASA - Ames Research Center)

Embryologist; former Project Manager of U.S. experiments on Cosmos flights (including Cosmos-1129); PI of frog embryology experiment for Spacelab-3.

Dr. Philip Teitelbaum (University of Illinois)

Physiological psychology; movement analysis; trained in use of Eshkol-Wachman movement notation system; vestibular specialist; brain-behavior relations revealed by postural adjustments and patterns of behavioral repetition.

Dr. Ingeborg Ward (Villanova University)

Behavioral endocrinology of reproduction; sex differentiation; neuroendocrinology.

Dr. O. Byron Ward (Villanova University)

Embryological indices of sexual differentiation; behavioral endocrinology.

Dr. John Vandenberg (North Carolina State University)

Behavioral ecology; olfaction and behavior in rodents and primates; endocrinology; population ecology

* * * * *

The author would like to express his sincere appreciation to these specialists, whose interest and enthusiasm embodied the ideals of commitment to science and scholarship.

Limitations imposed by time and funds precluded more extensive interviews with these scientists and, even more unfortunately, prevented the inclusion of numerous others. In particular, there is notable absence of input from specialists in behavior genetics, vestibular physiologists, specialists in perinatal physiology, computer instrumentation for movement analysis; kinesthesiologists; and representatives of various neuroscience specializations.

If anything, the experience of conducting this first round of preliminary interviews was a lesson in uncovering richness in intellectual resources and interacting with a diverse range of specialists about problems unfamiliar to them, but relevant to their expertise. The results of these initial discussions convinces me that they should be further pursued and can be used for productive, integrative results.

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PART II

MAMMALIAN REPRODUCTION IN SPACE

Although rats breed in diverse habitats all over the earth, we cannot assume that merely by putting a couple of rats in a habitable box they will produce babies in space. The Soviets have already made one such attempt; it is reviewed briefly below and then scrutinized in a later section of this report.

Despite the rat's marvelous abilities to adapt to new environments, they reproduce according to the basic "ground rules" of mammalian mating, and some of these rules may be violated directly or indirectly by conditions that prevail in space, such as zero gravity. Mating is based on the proper functioning of a complex system of behavioral and physiological events, both within and between individuals. This system may break down in space.

Indeed, general wisdom and results of the first Russian attempt both suggest that mammalian mating is vulnerable to disruption by space conditions. Fortunately, our extensive knowledge of how rats mate on earth may help us identify the disrupted components of the breeding system, devise ways of solving or preventing the problems, and design successful programs for maintaining normal reproductive function in space.

To examine both the vulnerability and the adaptability of mammalian reproductive behavior in a space station, I will cover the following topics:

1. History,
2. Behavior & physiology of reproduction in rats, with emphasis on potential problems in space,
3. A general approach to studying and accomplishing reproduction in space, based on an "ecological" analysis of the weightless habitat,
4. "New concepts" of design and design principles, and examples of how these concepts can be embodied in experimental space station habitats,
5. Specific studies and recommendations.

HISTORY: SOVIETS MAKE 1st ATTEMPT AT MAMMALIAN REPRODUCTION IN SPACE -- COSMOS-1129

In 1979, the Soviet Union launched Cosmos-1129 on an 18.5 day orbit. This was the fourth flight in the Soviet international program. They had decided to make the first attempt to mate mammals at null gravity. The Soviet decision was exciting and their approach bold. I call it the "Go-for-it" strategy. The design of the experiment was to fly a group of sexually-experienced rats and allow them to mate. At the end of the mission, females were to deliver, on earth, the first mammals conceived and gestated in an environment with no gravity.

The payload included five adult female rats and two adult males. They were housed together in a compartment (described below) that initially had a partition separating the male from the female rats. After five days in orbit, the partition was retracted and the seven rats were free to mingle and, of course, to mate. Females were expected to have as many as three estrous cycles during this cohabitation period -- plenty of time for successful reproduction.

On the ground, two groups of rats comprised the Synchronous and Vivarium Controls. The seven rats (five females and two males) in the Synchronous group were treated exactly like the rats in space. They were housed in a similar compartment, which was placed in a module like the one that was launched, and the rest of the animal and plant materials were similarly arranged for this ground-based simulation. Data on vibrations, noise, acceleration, impact forces, etc. were collected from the actual space flight and, after a five-day delay, were programmed into the Synchronous Control procedure. The Synchronous group received the same stimulation as the Flight group, except they remained at 1-G. The Vivarian Controls were maintained under typical conditions.

The results were most disappointing and are subject to various interpretations: None of the Flight females gave birth. In fact, it appears as though they may not have been pregnant. In addition, female rats in the Synchronous group, which never left the ground, also failed to demonstrate pregnancy. Vivarian controls appeared normal.

In all, the results of the experiment were "negative." These mammals did not mate successfully in space. Moreover, the failure of the Synchronous females to become pregnant suggests that there were additional problems with the procedure, beyond the effects of the space environment per se. I will discuss these possibilities in more detail later.

This is the brief and relatively non-illustrious history of

mammalian reproduction in space. Reproductive experiments have been conducted with non-mammalian life forms: plants, bacteria, insects, fish, frogs, and bird eggs. These have been more successful, but limited in scope. The future of mammalian reproduction in space remains a fundamental, unanswered question. The Soviets have gone at it first and made little progress. A U.S. space station would give NASA the opportunity to tackle some of the ultimate questions of biology in a manner that would make the problems tractable. The issues are: Will NASA take the opportunity and, if so, how shall we proceed?

**MAMMALIAN REPRODUCTIVE BEHAVIOR AS A DYADIC SYSTEM:
IMPLICATIONS FOR MATING IN SPACE**

STAGES OF THE REPRODUCTIVE CYCLE

Figure 1 shows a "stream" of events required for successful mating in rats. We will use this "systems" view to analyze the relationship between this earth-evolved phenomenon and its performance in a space station.

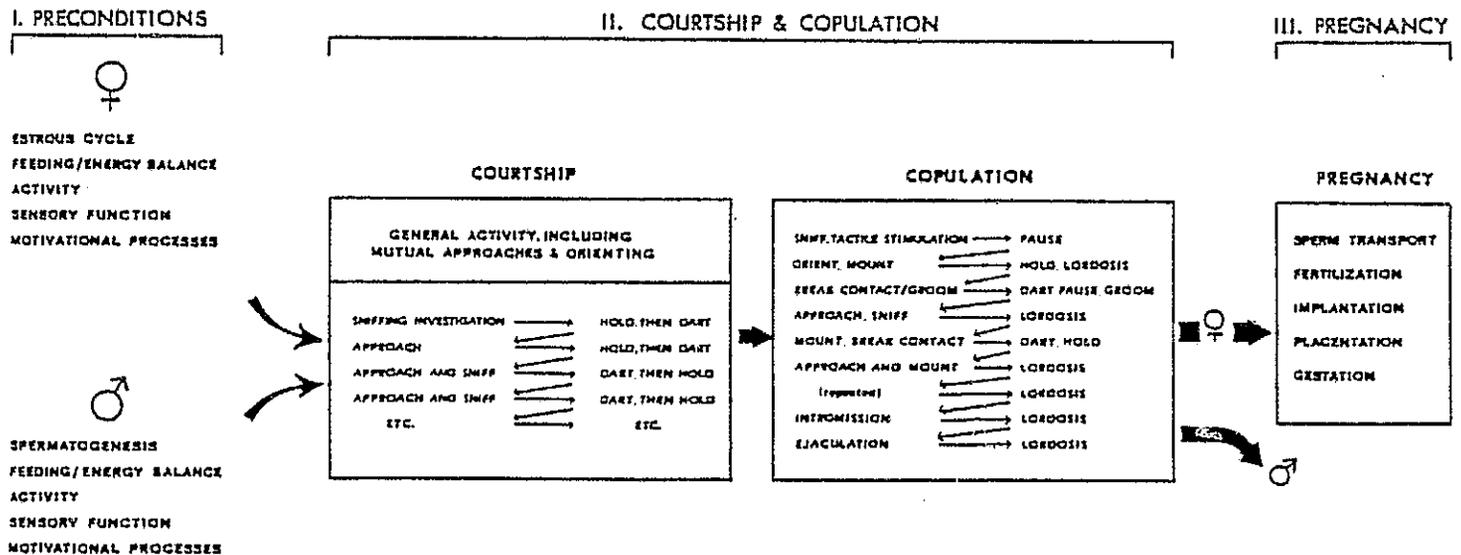


Figure 1. Reproductive behavior in the Norway rat. This depiction shows a "systems" view of the sequence of interactive events that comprise mating in rats. Stage I., Preconditions are intra-organismic conditions, necessary for the male and the female to enter subsequent stages. Stage II., Courtship & Copulation is the stage for crucial dyadic interactions. Stage III., Pregnancy involves the female, but is the eve of dyadic interactions with offspring.

The events and processes shown in Figure 1 will be used to make several important points:

(1) Mammalian reproduction is an integrated system comprised of specific sequences and combinations of behavioral and physiological factors;

(2) Mammalian reproduction is a dyadic system in which male and female are interdependent and interactive components ("Dyad" is from the Greek word "duos," which means "two.");

(3) The functional integrity of dyadic systems can break down, resulting in reproductive failure, if either the male or female is intrinsically disrupted or if the interactive mechanisms that coordinate them are disrupted; and

(4) The functional integrity of mammalian reproduction is susceptible to disruption by space conditions, but these breakdowns can be anticipated and prevented.

Figure 1 depicts rat mating as a dyadic system composed of parts that interact within orderly sequences, or stages. These stages, labelled along the top of the chart, will first be discussed as critical factors for reproductive success and then as mechanisms that link the stages together, forming a functional system.

PRECONDITIONS

The first stage in the mammalian mating system is the fulfillment of the necessary preconditions (Figure 2). These "intra-individual" factors reflect upon the health and normalcy of male and female. If any of these factors in either the male or female are significantly disrupted, the entire mating system can break down. Although this is not a complete set of preconditions for mating in the rat, it illustrates how these considerations are used for a systems analysis of behavior and the kinds of preconditions that must be taken into account.

1. Estrous Cycle. The female's estrous cycle, controlled by the pituitary gland, lasts 4-5 days. For one day during each cycle, the female rat is "in estrous" and is sexually attractive and sexually active. During the estrous phase, the female emits odors that attract the male. Subtle changes in her behavior further entice the male and help orchestrate interactions leading to copulation and fertilization. Any study of reproduction should test whether a weightless environment disrupts the estrous cycle. The procedure involves examining vaginal wash under a light microscope.

I. PRECONDITIONS



ESTROUS CYCLE
FEEDING/ENERGY BALANCE
ACTIVITY
SENSORY FUNCTION
MOTIVATIONAL PROCESSES



SPERMATOGENESIS
FEEDING/ENERGY BALANCE
ACTIVITY
SENSORY FUNCTION
MOTIVATIONAL PROCESSES

Figure 2. Preconditions for mating by rats. The necessary preconditions consist of similar factors for both sexes.

Estrous cycles are sensitive to daily rhythms of light and dark, of feeding, and even social contact. Sudden environmental change or stress can disrupt estrous cycles. Clearly, there are numerous indirect ways that space conditions, such as weightlessness, could halt or alter the female's estrous cycles. We know a great deal about the roles that odors play in maintaining the regular timing of estrous cycles. Dr. Martha McClintock at the University of Chicago, an expert on olfactory influences on fertility in rats and humans, agreed that social and environmental factors of animal housing could make the difference between reproductive success and failure (Appendix B). Subtle factors, such as the rate and direction of airflow in the animal's environment, must be evaluated before implementation. Maintenance of estrous cyclicity under all flight conditions should be studied early, using ground tests for proper procedural development.

2. Spermatogenesis. The secretion of testosterone and production of sperm -- the male zygote -- are hormonally-based preconditions for reproductive performance in the male. It is possible that space conditions could alter sperm production or testosterone secretion. At an informal meeting of U.S. and

Russian Cosmos investigators, the Soviets mentioned, anecdotally, that they found lower sperm counts in previously-flown male rats. If sperm production was diminished, fertilization could not occur.

As a parallel to monitoring female estrous cycles, sperm production, sperm motility, and the fertility potential of sperm should be routinely assessed. Related aspects of sperm delivery, such as a surge in luteinizing hormone (LH) that normally precedes a rise in plasma testosterone associated with copulation and ejaculation, can also be used as a metric of normal sperm production and delivery.

3. Feeding and Energy Balance. Adequate, well-regulated levels of feeding and energy balance are usually signs of healthy animals. The energetic demands of mammalian reproduction, particularly for females (pregnancy and lactation), raises these measures to primary importance. Stress may alter feeding and energy balance. To the surprise of my colleagues, previous flights have not included basic metabolic studies.

Most interviewees expressed disbelief that NASA had learned so little about animal life in space. They had assumed that there already existed a bank of information on basic issues, such as feeding, drinking, and related homeostatic processes in animals during and subsequent to exposure to null gravity. Without exception, they saw such data as vital for future work: Metabolic studies should make up part of any program of space research in the life sciences.

Relevant tests involve measuring food and water intake and monitoring dietary choices. Metabolic rates can be determined by measuring oxygen consumption, CO₂ production, energy excreted, and body composition. Body fluid and electrolyte measures are equally important. Most tests can be performed on earth, provided samples are properly collected and stored. Most desirable would be a "wet bench" facility for basic physiological measures on the space station itself.

4. Activity. Courtship and copulation in rats normally requires the expression of particular sequences of movements: approaches to specific body regions, hopping, darting, head movements, grooming, and more. If weightlessness disrupts the animal's activities by increasing, decreasing, or eliminating certain kinds of movements, or by inducing novel patterns of movement, then reproduction could suffer. Again, it was a shock for scientists I interviewed to learn that neither the U.S. nor the Soviet Union collected systematic visual records of the quantity or quality of animal movement during and subsequent to space flight. Long-term effects are most pertinent, of course, and here a space station would fill the bill.

In the Introduction, I noted the special skills available from behavioral biologists that pertain to space-relevant movement analyses. I would rate such studies with first-level priority. They should begin on earth and, if possible, exploit the upcoming Space Lab missions. This work is non-invasive, can be conducted simultaneously with most other tests, and will provide valuable data to numerous other lines of investigation. For example, metabolic studies cannot be interpreted fully until we can relate metabolic expenditure to activity in space (see interview with Dr. Schallert, for example).

Careful analysis of the amount and kind of activity is also in the interest of the animals themselves. Based on simple visual records, much can be learned about their well-being and particular space-related needs. We will return to such issues later in this report.

5. Sensory function. Throughout the reproductive cycle, a variety of sensory cues -- visual, auditory, tactile, vestibular, proprioceptive -- coordinate the stream of responses between male and female. Although all cues play a role, some, such as odors, are more important than others: their absence can disrupt the dyad.

Long-term exposure to space conditions could alter sensory function. Space station studies will provide valuable data on this issue. Proper sensory function in both male and female rats is an important precondition for mating because it allows each participant in the dyad to recognize the gender and reproductive status of the other and then to coordinate their interactions. The odors of estrous females, for instance, arouse the male rat, increase his level of activity, guide his investigations of her, and then guide his courtship. Likewise, male odors attract females and signal his sexual competence. The male's precopulatory surge of LH, mentioned above, is an odor-induced hormonal reflex. During copulatory sequences, rats use high frequency vocalizations (approximately 22 kHz) to coordinate their interactions.

Disruption of normal mating can be caused by sensory impairment, a condition only indirectly related to sexual function per se. Fortunately, there are adequate methods for sensory testing in rats. Some refinement, accomplished through ground-based research, is needed to make these tests efficient and applicable to sexual function, but this could be accomplished with existing approaches.

Again, it would be an important precaution to evaluate flight habitats for the possibility that they might contain features that obscure important mating cues. I have already mentioned the possible effects of airflow on odor cues.

Background noises could "jam" the frequencies used by rats to communicate.

6. Motivational processes. Most of the important categories of mammalian behavior -- feeding, drinking, sex, parental behavior -- involve an aspect of behavioral control referred to as "motivation." Difficult to define but essential to recognize and consider, motivational processes involve the factors (usually within an organism) that affect its responsivity to its environment. In the context of rat sexual behavior, we have noted the importance of reproductive competence and sensory function. But for mammals, these preconditions must be accompanied by an additional factor -- the motivation to use its signals and skills to achieve mating.

Once again, contemporary biobehavioral science is equipped to assess and to measure sexual motivation at many levels. For instance, we have separate tests to evaluate whether a male rat can detect an estrous odor, whether he can discriminate it from other female rat odors, and whether he is motivated to respond preferentially to it. For both male and female rats, we have quantitative tests that measure levels of sexual responsivity. In females, a score called the "lordosis quotient" (LQ) is used to evaluate the strength of her receptive responses to the stimulation she receives from a sexual mount. The LQ varies with many factors, such as hormone level, experience, and environment. In sum, there are an array of biobehavioral tests, well suited for the evaluation of behavior in a space station and for the diagnosis and solution to space-related problems of mammalian function and well-being.

COURTSHIP AND COPULATION

Any plan to mate rats in space must consider the social, behavioral, and physiological dynamics of Courtship and Copulation (Figure 3). The following account provides a glimpse of the complex and finely-tuned interactions involved in mating on earth. We will then evaluate results that could occur in space.



Figure 4. A male rat engaged in a sniffing investigation of a female. This is a stereotyped interaction. Though simple in appearance, the investigation involves the coordination of behavioral timing by female and male. The female solicits the male's approach with her movements. Her pause permits contact. The male directs his behavior toward her genital region. His light touch signals the female to remain immobile. Sniffing increases arousal and leads them through the courtship sequence.

II. COURTSHIP & COPULATION

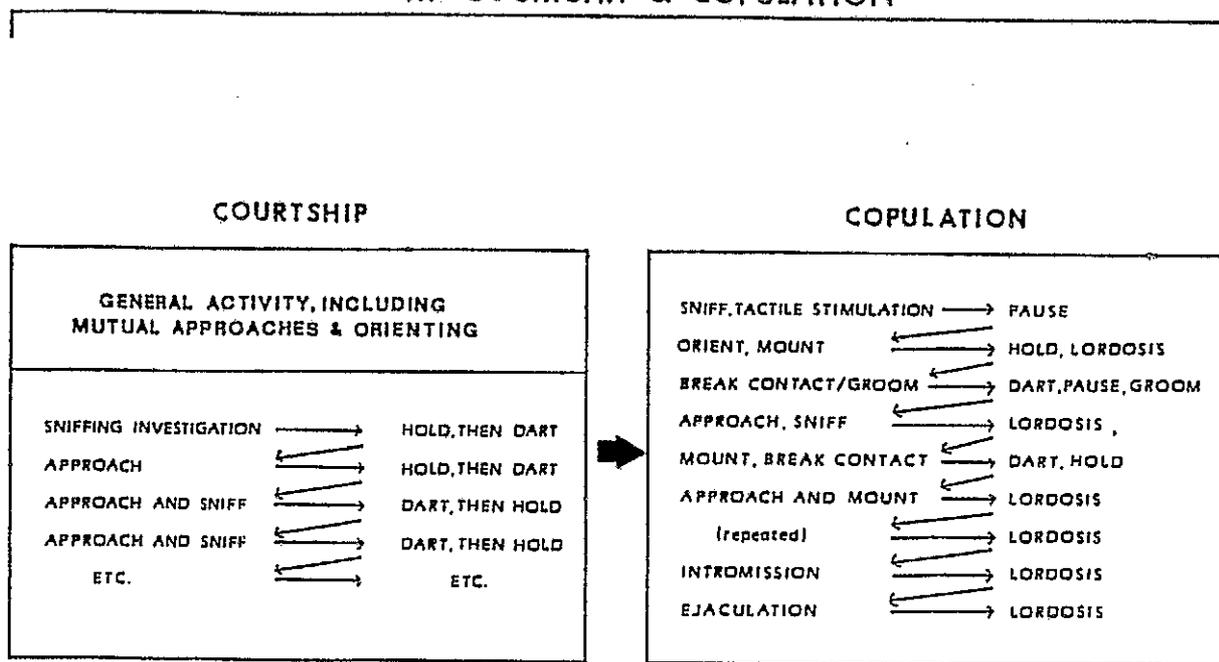


Figure 3. Courtship & Copulation. Stage II of the reproductive cycle of rats. The diagonal arrows within each box indicate channels of reciprocal interaction, forming chains of behavior. The boxes are linked by behavioral transitions.

1. Approach/Investigation. On the day of estrous (sexual responsivity), the female rat produces vaginal odors that arouse the male. Initially, the estrous scent increases the male's activity in a general manner: He walks, turns, stands, and sniffs more than usual. The movements tend to bring him into proximity of the estrous female. The male then approaches and sniffs her body, concentrating increasingly on her genital region. Figure 4 depicts the stereotyped posture of these sniffing investigations.

2. Darting/Pausing. In response to the male's approaches, the female usually moves away with sudden "darting" movements. These stereotyped movements are characterized by rapid acceleration sometimes so energetic that she appears to hop along. Then the female pauses in a manner that apparently entices the male. He approaches. She darts. He approaches and sniffs. She darts away. The male approaches again, and the sequence repeats again (Figure 3).

The approach/darting sequence helps orchestrate the male and female. Their levels of arousal tend to synchronize and their activities become coordinated. When the female darts, the male follows closely and her pause becomes the signal for mounting. Figure 5 shows the mounting postures of the pair. This is a critical event.



Figure 5. Sexual mounting during copulation. The copulatory sequence in rats involves repeated mounts, separated by pauses during which the male grooms and again approaches and mounts the female. Tactile stimulation from the male elicits the "lordosis" response in the female.

3. Mounting. As the male mounts the female, his body provides a pattern of tactile stimulation that elicits lordosis, a reflexive postural response characterized by immobility, raised head, arched back, deflected tail, and elevated pudendum (Figure 6). Lordosis positions the vaginal area for penile intromission and thrusting. Thus, the approach-mount sequence evolves into one that includes intromissions and thrusting. Copulation consists of repeated series of approach-mount-intromission sequences until a sequence culminates in ejaculation.



Figure 6. Lordosis in a female rat. This posture is normally seen only with tactile stimulation to the female's haunches, such as that provided by a male preparing to mount. Lordosis is displayed while the female is stationary. It is necessary for intromission.

Ejaculation is accompanied by a prolonged thrust, with the male's pelvis held against the female. He then leaps backward, sits on his haunches, and grooms his penis. After a refractory period of several minutes, he may begin the sequence again, continuing this pattern for an hour or more. Males ejaculate during only about 20% of the sequences.

This description of complex interactions on the behavioral level only hints at other levels of analysis. The multiple intromissions required for ejaculation, for instance, also provide the female with cervical stimulation that elicits a neuro-endocrine response necessary for ovulation and, thus, fertility. The female's posture during copulation helps the male deposit a sperm plug that fits closely in the cervical/vaginal canal and thus stimulates mechanical and chemical transport mechanisms which, in turn, influence the likelihood of fertilization.

4. Group mating. We have reviewed mating between single animals. In their natural habitats, however, both domesticated and wild strains of Norway rats mate in groups. Males take turns copulating with estrous females, repeatedly changing partners before ejaculating. Females take turns soliciting males and eventually receive ejaculations as well as intromissions from all males in the group.

These "promiscuous" copulatory patterns are optimal for neuroendocrine function. For males, intromission every two-three minutes minimizes the number needed for ejaculation. For the female, 10-15 minutes between intromissions is optimal, maximizing sperm transport and inducing a progestational state. When rats mate in pairs, intromission occurs every minute, an interval much shorter than the optimal for either sex. Group sex, however, frees both male and female to mate in their sex-typical patterns.

Social dynamics within the group helps regulate male and

ORIGINAL PAGE IS
OF POOR QUALITY

female mating behavior. Male turn-taking, for instance, permits each male to copulate at optimal intervals and mate with all the estrous females. After one male completes one or two ejaculatory series, another will respond to female solicitations, but only after approaching the previous male. Females also take turns, alternating solicitations.

PREGNANCY

Ejaculation marks the end of the copulatory phase and heralds the beginning of the pregnancy phase (Figure 7), which begins at fertilization and lasts until delivery. On earth, pregnancy lasts 21.5 days.

III. PREGNANCY

PREGNANCY

SPERM TRANSPORT
FERTILIZATION
IMPLANTATION
PLACENTATION
GESTATION

Figure 7. Pregnancy in the rat. Stage III of this "systems" view consists of a set of separable factors that might be vulnerable to space conditions.

1. Sperm Transport. I have already mentioned the phenomenon of sperm transport because it is a "spill-over" from the copulatory sequence. The male rat's ejaculatory thrust helps install a vaginal plug in the female that mechanically and chemically facilitates movement of the sperm through the uterus. Without a properly positioned plug, the uterine environment is less conducive to mobility of the sperm, and the probability of fertilization is reduced significantly.

2. Ovulation. In order to have female gametes available for fertilization, ovulation must occur. Ovulation involves the release of ova (eggs) from the ovaries, where they reside from

infancy. The full, lifelong set of oocytes are formed during the females' early development, as part of differentiation and maturation. Again, copulatory events contribute to this early step in pregnancy, particularly in coordinating its timing with respect to sperm deposit. The alternating, turn-taking patterns of group mating facilitate this timing.

3. Fertilization. Presently, there has never been an attempt to achieve fertilization of vertebrate zygotes in space. Fertilization in space has been accomplished with bacteria and fruit flies, in both U.S. and Soviet experiments.

4. Cleavage. Cleavage is characterized by the movement of subcellular elements to opposite sides of the egg. Some scientists think this polarization is controlled by gravitational forces.

Dr. K. Souza (NASA-Ames) will use frogs eggs, which develop outside the mother's body, to test this hypothesis on Space Lab. Should gravity control cleavage, Souza will have made an important contribution to a long-standing inquiry in developmental biology (see Appendix J).

5. Implantation. Growth begins when the egg invades the uterine wall between the 3rd and the 5th day of the Embryonic stage. Implantation requires a complex of intercellular events, which may be disrupted by gravity. Again, this is an example of a stage of vulnerability. We simply do not know what to expect.

6. Development of the placenta. The placenta, which links the embryo to the mother, develops the second week after fertilization and permits transport through the blood system of oxygen and nutritive elements. Thus, changes in the mother's cardiovascular or digestive systems might interfere with the embryo's development. Biopses of flighted rats have shown unusual profiles of blood serum amino acids, which suggests that the embryo's profile might be affected as well.

7. The fetal period. By the eleventh or twelfth day after conception, the early formative processes are usually sufficiently advanced that the conceptus is a full-fledged fetus. The fetal stage involves the development of organs, bones, and the nervous systems.

The sequence of events that occur in the fetus during the last half or final trimester of pregnancy are dramatic and pertinent to issues of reproduction and development. I will, however, review fetal maturation in the section of this report concerning development, which will aid the internal continuity of this section.

During pregnancy, unlike the copulatory and postnatal phases

of reproduction, much of the really dramatic action is hidden from view. There are, however, a few important, observable events during pregnancy which can be measured by non-invasive, non-disruptive, direct methods. Three important aspects of normal (terrestrial) pregnancy in the rat are changes in body mass, energy balance, and self-grooming.

CAN RATS MATE IN SPACE?

With this rapid introduction to mating rats, let's consider the question of whether it is possible for rats to mate in a space station. We will approach the question by considering what will probably be involved in such an accomplishment.

Figure 8 depicts the situation that we -- and our rats -- are likely to face if these animals are simply placed in a compartment and expected to mate in space. We have reviewed



Figure 8. Artist's depiction of adult rats floating weightlessly. Coordinated social interactions such as mating might be difficult under such conditions, but we can formulate solutions to the problem.

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enough about the physical and social dynamics of mating that the reader should anticipate the myriad obstacles to successful mating in space. Let's consider a few aspects in detail.

Zero-gravity may effect the amount and kind of locomotor activity. We have discussed this general question in the context of "preconditions," but the threat posed by activity alterations is echoed here, perhaps even more intensely. The role of the female's darting, hopping, and pausing is important in the coordination of copulation. Locomotory control is needed. The male's sniffing investigation requires a pause by the female and a precise orientation by the male, one that can be maintained long enough for him to give and receive thorough sniffing and licking investigations.

Figure 9 is a composite showing mounting and lordosis postures. The arrows depict points of anti-gravitational support that are normally part of the coordinated muscular/postural mechanisms used during normal, terrestrial mating. What will happen when anti-gravitational support is absent?

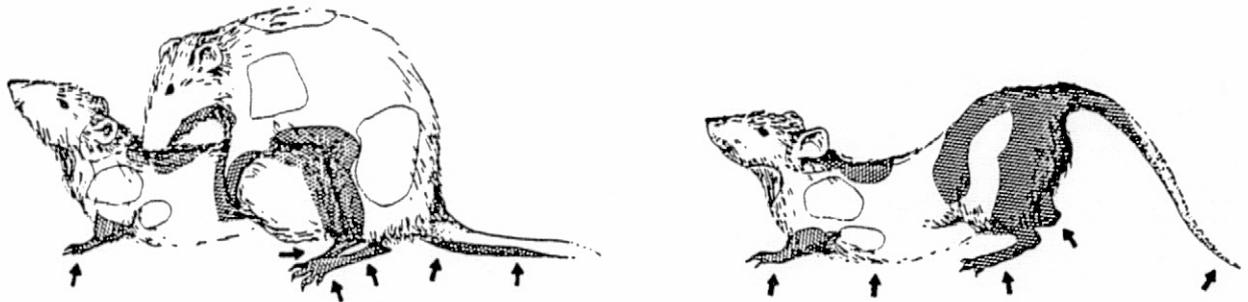


Figure 9. Zones of proprioceptive and tactile stimulation during copulation. Shaded areas show the areas of proprioceptive feedback, providing the rats with information about the location and angles of their own body; such cues are crucial for maintenance of precise postures. The cross-hatching shows areas of tactile stimulation, vital for eliciting lordosis, ovulation, and ejaculation.

The grey zones shown on the rats' bodies depict likely areas of proprioceptive information, crucial points that normally give the animal feedback about the location and angle of the body with respect to itself and its partner. Such feedback is part of postural maintenance and is another possible challenge of mating under weightless conditions.

The dark-shaded zones show areas of tactile stimulation. These portions of the rats' body surface are normally stimulated during courtship and copulation, and this stimulation has been shown experimentally to be important to both male and female for the maintenance of postures, coordinating copulatory patterns, and for the achievement of ejaculation and ovulation. If movement patterns change the amount or patterns of tactile stimulation, then reproductive success may suffer. Again, detailed observational data is absolutely essential.

Finally, it should be repeated that group mating is the optimal situation for the rats, in terms of individual adaptation and efficient husbandry. Thus, NASA should anticipate the use of group housing for rats and other rodents used for research on a space station, particularly when efficient breeding and husbandry is important. This plan brings with it the need for careful and systematic studies of group dynamics. These studies should first be conducted on earth to establish good normative data for comparisons under identical arrangements in space. Part of this effort should include studies of groups in habitats that differ in configuration. We will return to discuss some examples of alternative habitats in an upcoming section.

CRITIQUE OF THE SOVIET'S COSMOS-1129 RAT EXPERIMENT

In an earlier section, I reviewed briefly the plan and the results of the Soviet's Cosmos-1129 flight. This pioneering flight, conducted in 1979, represents space biologists' only attempt thus far to study mammalian reproduction in space. The Soviet's 1983 flight will be the second attempt. Because the history of the problem is so limited and because it has been carried out solely on Soviet flights, I have repeatedly mentioned these studies. Fortunately, NASA has maintained active, cooperative ties with the Soviet's Cosmos program and U.S. investigators have participated in several flights.

The first-hand experiences of Americans who worked in Moscow on Cosmos-1129, such as Dr. R. Keefe (BioSpace, Inc.) and Dr. K. Souza (NASA-ARC), are important avenues of benefit from the flights. I have interviewed these and other scientists. In addition, as a U.S. investigator on the Cosmos '83 flight, I have observed and spoken with Soviet scientists who designed and conducted their rat studies.

The following critique of the first Soviet attempt to mate rats in space is based on my analysis of published reports, first-hand observations, and interviews. Some of the information presented is unique and important. In particular, the discussions with Dr. Richard Keefe (Appendix D) provide

valuable additions to the published information on that historic experiment.

Recall that the Cosmos-1129 experiment was planned as a mating experiment. Five female and two male rats were flown for 18.5 days (see History section, above). Two control groups were used on earth. The Synchronous Controls were housed and handled identically to the Flight Group, including housing in a mock satellite, but they were kept at 1-G. The Vivarium Controls were handled according to typical, terrestrial procedures.

Neither the Flight nor the Synchronous females showed clear indications of pregnancy. No litters were produced. Despite negative results, a simple video tape would have provided a wealth of information. Incredibly, no observational data of any kind was recorded. We can therefore only speculate about what went wrong.

Figure 10, drawn from a photograph in NASA's report, shows the compartment that housed seven rats. The disc shapes on the walls represent food delivery mechanisms. Smooth, narrow rods run lengthwise over eight troughs that collect urine and feces. The ceiling was made of opaque plastic and perforated with small holes for ventilation. On the fifth day of flight, the partition separating males and females retracted, permitting free interaction. Unfortunately, no U.S. investigators were permitted to examine the compartment after flight to determine how well it functioned.

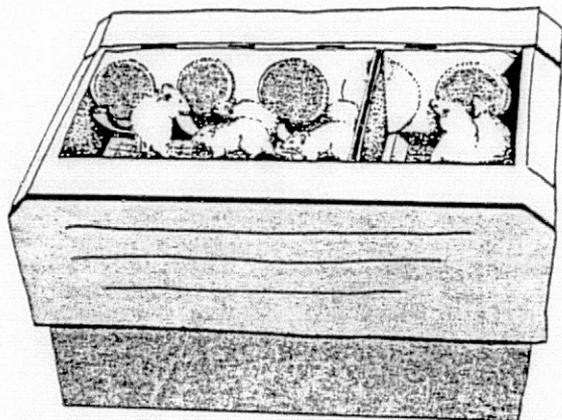


Figure 10. Drawing of the rat compartment used on the Soviet's Cosmos-1129 biosatellite. The compartment housed seven adult rats. Food was presented through mechanisms on the chamber walls, depicted by the discs. The drawing is based on a photograph in NASA's technical report of the Cosmos experiments.

The Soviet's rat compartment was similar in size and configuration to a rodent cage on earth. It was 65cm l x 20cm w x 16cm h. While in orbit, the weightless rats would float around freely unless they could hold onto graspable surface.

Graspable surface. If weightless rats are to remain stationary or engage in social activities that require oriented movement and body contact, they need surfaces to hold or move against. In the Cosmos compartment, unfortunately, only the floor and some portions of the feeding ports were graspable -- a surface area of 1,300 square cm -- and even these weren't optimally designed. The bars on the floor, for instance, were smooth and widely spaced and ran in only one direction. This surface probably made the animals orient perpendicular to the bars.

Only 20% of the compartment surface could be grasped and used for controlled movements, such as those in courtship and copulation. I suspect that the rats floated most of the time. As I have shown, copulation in the rat is based on the ability of the male and female to make and break oriented movements. If rats mate in space, it may only be in special environments that are designed with spatial features and configurations that reinstate or replace cues, such as surface contact and support along their bodies, that are normally subserved by gravity.

Feeding regime. Rats normally eat during the shorter, darker period of the day, a temporal pattern apparently determined by circadian rhythms.

On Cosmos, the paste diet dried into a brittle mass if exposed to air for prolonged periods and was therefore presented every six hours through the "day" and "night." Disrupting the light/dark feeding cycle may have disrupted other physiological processes, such as the estrous cycle, and thus prohibited fertilization among both flighted and control animals.

In addition to changes in the light/dark feeding cycle, the abrupt change to a paste diet may have disrupted the estrous cycle, which is also normally correlated with the animals' daily rhythm of activity and feeding. Thus, the six-hour feeding regime may have produced a pattern of behavioral interactions that interfered with courtship behaviors in the rats.

Extraneous light. The light/dark cycle is one of the most powerful determinants of estrous cyclicity. Dr. J. R. Keefe, an American participant in the Cosmos flight, believes the light from other animal compartments might have leaked into the mating compartment, causing continuous illumination and thus prohibiting ovulation (see Keefe interview, Appendix D).

Partitioning the compartment. The metal partition that separated the males and females during the first five days of the flight may also have prohibited the free movement of individual cues, such as odors, that help animals establish and maintain familiarity. Female rodents are especially sensitive to unfamiliar animal odors, which may have subsequently disrupted estrous cyclicity and pregnancy (see Appendix B).

Conclusion. Our review of the Cosmos protocol reveals several factors that can account for the disappointing outcome. The most important lesson, however, is that conditions of space flight can disrupt the intricacies of reproduction, and we must therefore prepare an appropriate support system. To do so, however, requires careful monitoring before, during, and after flight. Most important is the collection and analysis of detailed behavioral data. Real time as well as time-lapse records are both necessary. We face numerous gaps in basic, but crucial information, much of which can be obtained with little difficulty and minimal expense.

A PROGRAMMATIC APPROACH TO REPRODUCTION IN SPACE

WHY NOT JUST "GO-FOR-IT"?

It could be argued that the most expedient approach to achieving mating in rats in space is to plan a mission which simply gives them the opportunity to reproduce ... and "go-for-it." This is, of course, the approach used previously by the Soviets. Although this bold strategy has some attractive features, such as the possibility of rapid success, I believe that we should not be seduced by the promise of easy results. First, note that the Go-for-it Strategy is the one that generated the disappointing experiments on Cosmos-1129. I have contended that the major disappointment of the Cosmos-1129 experiment was not that the rats failed to produce offspring, but that we learned much less from it than we could have, had additional measures been utilized. Instead, we have no direct data to help us identify the reason(s) the Cosmos rats failed to reproduce, nor did we gain much data on the effects of the flight on these animals, even though they are among the few mammals that have been flown.

It is also important to distinguish between conditions that are sufficient to obtain mating and conditions that are either optimal, difficult, or something in between. In other words, it will be scientifically incomplete to cease empirical studies of mating, once reproductive success in space has been obtained. Achievement of successful mating does not insure that we understand the processes, or that such matings are normal. In particular, we will want to evaluate carefully the fecundity of animals in space, and the quality of their offspring and that of subsequent generations. Thus, we need precise measures of reproductive efficiency. Some of these measure can be borrowed from terrestrial methods. Others will have to be devised and tested in space. On earth, for instance, ecologists are concerned with the "energy budgets" of reproduction -- the number and types of calories involved in producing offspring. Careful metabolic and behavioral studies are used to evaluate the costs of reproduction to males and females during various phases of the reproductive cycle. In these regards, we will

want to know more than whether the rats can mate. We must compare reproduction in space and on earth in terms of energetic costs, efficiency, rate, and characteristics of offspring. The results of such analyses will provide insights into the fundamental nature of reproductive processes on earth, open new vistas in understanding evolution of reproductive and developmental strategies, and help design efficient animal resource facilities for space research for years to come. For pure and practical reasons, these are important, seminal studies.

The resounding failure of the first attempt by the Soviets to mate rats in space is a valuable lesson to all researchers: Successful management of animal studies in space is a formidable task, requiring appreciation of a melange of crucial factors, some striking and some subtle. All of these factors compose the biological system of sexual reproduction that has been fashioned by natural selection over millions of years to function on Earth. To remove this finely-tuned system from the major environmental forces of the earth, to expose it to the unique, un-earthly conditions of space, and yet to preserve its functional integrity is a challenge to our ingenuity and a test of our understanding of life processes.

In all, what is called for is a programmatic approach to the analysis of mammalian reproductive behavior in which its various components and stages are analyzed separately and then synthesized to function as an integrated system. This approach is exemplified by the forthcoming analysis of Reproduction and later, of Development. In both cases, I discuss the context (habitats) of the animals and the functional units that comprise male-female as well as mother-litter dyads.

In the present section of this report, I discuss the structure and contents of such an approach. The program serves dual purposes. First, it is goal-oriented. Simultaneously, each stage contains research projects which can also be treated as stand-alone studies, as well as sources of information valuable to the needs and interests of other life science enterprises. For example, I shall discuss the design and testing of various rat habitats in relation to reproductive activities. The observations and tests involved in these efforts will be directly applicable to nearly any other project in which animal caging is required. Data on movements and postures collected in studies of habitat utilization by the rats could be used for neurological studies of vestibular and cerebellar function. Data collected on feeding and activity patterns could serve as the foundation for work by nutritionists and specialists in chronobiology.

The reproductive sequence depicted in Figure 1 shows the stages as inter-connected and interdependent. They can be studied as units and then studied in relation to each other.

Discussed below are aspects of this strategy. For this presentation, I have chosen a subset of the possibilities that illustrate the approach that I recommend. These are my examples are not intended to represent an exhaustive set of important questions. If NASA agrees that such an approach should be examined intensively and developed into a coherent plan, such an effort can be organized, but it is beyond the scope of the present report, which is designed as a review and hopefully as a tantalizing appetizer to herald greater and more intense efforts.

THE HABITAT: ECOLOGICAL ANALYSES OF WEIGHTLESS WORLDS

I interviewed Dr. Chris Schatte (Project Scientist, NASA-ARC) and Dr. Richard Keefe (Cosmos PI, BioSpace, Inc.) about the sources and rationales for the designs of the rodent flight cages used by NASA and in the Soviet Union. Neither of these experts was certain of the answers. In fact, they both guessed that clear answers were probably unknown. Schatte suspected that some anonymous engineer(s) in a company such as Lockheed probably planned the U.S. compartments with little specific guidance. Similarly, Keefe ventured that the Russian design was simply inherited from an earlier flight compartment used on a mission before the U.S. joined the Cosmos series. It is instructive to note that in neither country has primary importance been given to the configuration of these cages from a scientific point of view.

1. Current Practices Reflect "Geocentrism." Standard guidelines for the maintenance of laboratory animals prescribe cage sizes, based on measures such as square inches per unit body weight. In addition, there are recommended heights for cages, intended to give freedom of movement. These guidelines, and the principles that fostered them, are designed with the welfare of the animals in mind. One of the additional benefits of adherence to such standards for the animals' welfare is that the quality of scientific use of the animals is almost always enhanced. Stressed, unhealthy animals from miserable environments tend to yield data commensurate with their condition.

I believe that it is probably a serious error to use guidelines for terrestrial cages in the design of flight cages or habitats. I believe that both the animals and the science are likely to suffer. Automatic adherence to standards based on terrestrial conditions can be termed "geocentric." We must free ourselves from geocentric attitudes and devise approaches to designing flight habitats that are best for the animals and for our scientific purposes. In most cases, these two sets of interests are identical.

The design of flight habitats and test environments and thus the success of future animal experiments can be enhanced by drawing more extensively upon knowledge gained from ground-based research. We can reject the practice of geocentric methods, but adhere to the principles of animal welfare that they embody.

The approach that I recommend is to consider carefully the features of organism-environment relations that support the animals' well-being, and then design flight environments that provide such features under conditions that prevail in space. Most of these issues are not resolvable at the drawing board. Most will have to be answered empirically. I can, however, discuss the kinds of approaches and tests that warrant considerations, for they are sufficiently important to be included in plans for a space station. These recommendations are offered to exemplify the kinds of contributions that can be made by areas of scientific specialization that have not yet been exploited by NASA.

2. Habitat Surface/Volume: A Critical Metric for Compartment Architecture. I propose that the ratio of surface area to volume (S/V) of an animal cage (habitat) is an important, behaviorally-significant metric in a weightless environment. Habitat S/V can be used to describe the functional attributes of any three-dimensional environment and provides a common metric with which to compare and evaluate different habitat configurations.

What does Habitat Surface/Volume mean in behavioral terms? Let's analyze the rat compartment used on Cosmos-1129. The Soviet rat compartment (Figure 10) is a parallel piped, basically a shoe box design. It is 65cm l x 20cm w x 16 cm h. Thus, the total space within the chamber (Volume) is 18,800 cubic cm. The total area of the internal boundaries (Surface) is 5320 square centimeters. While in orbit, the weightless rats would be free to float anywhere within the compartment's volume. Only one portion of the internal surface -- that used for waste collection -- was not smooth and consisted of rods that could be grasped. These rods, however, were on turrets that moved and may not have been good for long-term support and control.

The rat compartment of Cosmos-1129 was a geocentric's creation. It adhered to a wall-floor-ceiling configuration, even though such functional asymmetries do not apply at null gravity. The Habitat S/V ratio was 5320:18,000, or 0.28. Actually, from the rat's point of view, there was only one graspable area (part of the floor) and that was less than 1300 square centimeters. Thus, the functional, graspable Surface/Volume ratio of the Cosmos habitat, was probably closer to 0.07!

I think that we can -- and must -- do much better than Habitat S/V's of .07 - .28. Consider the following examples and then the alternative approaches they suggest. For purposes of comparison, let's examine the S/V of habitats or shapes, each with a volume of 20,000 cubic centimeters (a little larger than the Cosmos compartment). Each is the same "size" in terms of volume but differs in configuration. They are shown in Figure 11. There is: (A) a cube, (B) a sphere, (C) a parallel piped, (D) a parallel piped with the same volume as "C" but with height and width reduced by half, (E) a cylinder, and (F), a cylinder containing a helix. The ratios of S/V of each are given below each drawing.

The differences are impressive. The S/V ratios range from .18 to .80, with equivalent volumes. Larger ratios of Habitat S/V mean that the rats have more control over their orientations and movements in the environment, because of increased contact with surfaces, via their paws, tail, and other body parts. I predict that such habitats with larger S/V's will be less stressful to animals such as rats, that they will adapt to the conditions in them more readily, and that they will therefore be more likely to display normal biological processes, including reproduction, as a function of environmental quality.

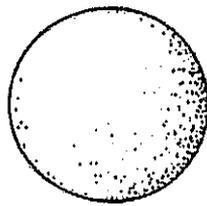
Habitat S/V appears to me to be a new and valuable concept for the design and evaluation of space habitats. I will expand on some of these ideas later.

3. Visual Surveillance. To me and to many of the scientists I interviewed for this project, the lack of detailed visual records of the behavior of rodents during spaceflight is one of the most serious and disappointing omissions of past research projects. It appears that space scientists have not appreciated that behavioral data can make important contributions to biological research. Nevertheless, recent Soviet expressions of interest, NASA's support of U.S. behavioral studies for the Cosmos-1983 flight, and, indeed NASA's selection of the proposal that described the goals of this report, suggest that the climate is changing. And well that it is. It is essential that the design of future flights include the collection of data on the behavior of the animals at all stages of flight.

"Behavior" in this context has multiple, broad meanings. Behavior can be recorded at many levels; each has its own advantages. Fortunately, from the same record of behavior, we can often extract measurements from different levels of analysis. For instance, from my video tapes of mother-litter interactions of rats that will be part of the Cosmos-1983 flight, I will measure "molar" behavioral patterns, such as maternal presence in the next over 24 hour periods and circadian rhythms of feeding, nursing, and activity. In addition, we will also conduct relatively "micro-analyses," such as the licking that

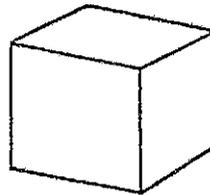
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the mother directs at the anogenital regions of the pups and the number and distribution of her milk letdowns during lactation.



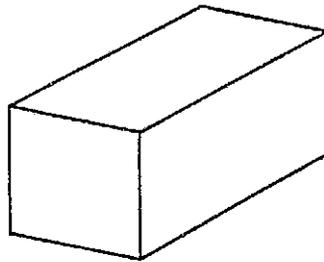
A

$$S/V \cong .18$$



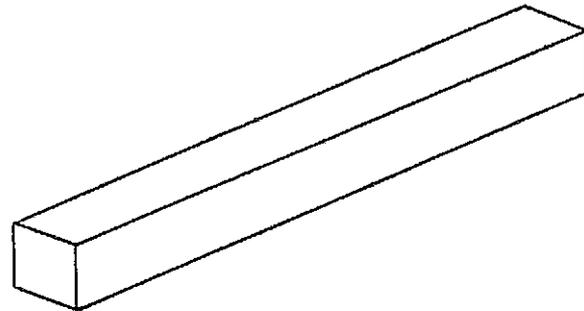
B

$$S/V \cong .22$$



C

$$S/V \cong .35$$



D

$$S/V \cong .67$$



E

$$S/V \cong .67$$



F

$$S/V \cong .80$$

Figure 11. Geometric forms analyzed for ratios of surface area / volume (S/V), shown below each form. The calculations were based on each form having a volume of 20,000 cubic cm. in order to compare the S/V across each alternative. Larger S/V values represent three-dimensional spaces in which more surface area for tactile stimulation, support and grasping would be available. S/V is a useful metric for objective comparisons in habitat design.

Important aspects of individual behavior during flight to study also include posture, orientation, coordinated limb movements, speed and accuracy of movements, analyses that dissociate phasic and tonic aspects of movement. Behavioral categories such as feeding, drinking, self-grooming, and exploration must also be quantified and compared to terrestrial patterns. In addition, group and social behavior must be similarly analyzed. This is particularly important to the goals of conducting studies of reproduction and development.

Enormous progress has been made in biobehavioral analyses with the application of movement notation systems. These have been used with animals (mice, rats, wolves, and humans) to study the effects of drug treatments on nervous system function, the analysis of vestibular and proprioceptive integration, effects of brain damage in infant and adult organisms, and patterns of social interactions among groups of animals. Computer support (hardware and software) has now been implemented to facilitate this work.

Investigators using these systems of movement notation are in a camp of creative and exciting scientists. Their interests are broad and significant. I have interviewed several for this project and they all expressed enthusiastic interest in the prospect of space station research. Without exception, they agree that visual records are extremely important and that numerous other lines of research will be thwarted, if not eliminated, unless basic behavioral analyses are conducted. Such studies are relatively inexpensive, particularly since many different studies can be performed on the basis of the same video tapes or films (which can be copied and distributed). In addition, this work is noninvasive and can proceed without interfering with simultaneous studies.

Conclusions: Cages, compartments, and habitats must be designed so that animals can be observed by camera. In addition, other engineering techniques of movement sensing can be used to collect additional behavioral data. Primary importance must be given to habitat design that permits relatively unobstructed visual surveillance. It should be anticipated that video cameras capable of real-time, slow-motion, and time-lapse recording will be used within any of these habitats. The recording technology is available. Visual observations can also be conducted through light-dark cycles. These aspects of design and research deserve the highest priority.

4. Surface characteristics. I have already referred to the characteristics of the internal boundaries of the habitat. (In a terrestrial habitat, I would use the term "substrate," but the prefix "sub" is a geocentrism that we must avoid.) One of the most important characteristics of the surface of the weightless environment is the extent to which it offers the inhabitants an opportunity to maintain or control their movements or orientation.

Rats can use their paws for grasping. Figure 12 shows paw

prints of an adult rat. The forepaws are smaller, about 7mm palmar width; the digits are no longer than 6mm. Rear feet are larger, about 3 cm long, with 9mm digits. With these paws (front and back), rats can easily grasp by bending the digits down against the palmar surface.



Figure 12. Paw prints of an adult rat. Note the relatively long digits that the rat can use for grasping. The larger prints are of the hind feet.

A functional surface for a rat habitat at 0-gravity would be designed to enable the rat to use it for control and orientation. The surface should be constructed in a manner permitting the animal to use its paws for grasping. Bars, mesh, rough texture or penetrable materials could be tested. On Earth, the rat uses its tail for leverage, support, and balance in many situations. It is a sensitive and functional appendage. Surface features of a functional habitat would not obviate such advantages.

5. Temperature and Texture. The temperature of the compartment surface will probably affect the animal's behavior. Rats are called a "contact species" because their terrestrial behavior is dominated by activities that involve direct cutaneous contacts: They "huddle" with other rats, rest and sleep in piles, and show a marked tendency to stay near vertical and overhead surfaces, a behavior called "thigmotaxis." It would probably be possible to affect the manner in which the habitat was utilized by controlling the textures and surface temperatures.

6. Feeding Schedules. I have criticized Soviet use of a 6 hour feeding schedule. I recommend continuous access to food and water, with monitoring of the patterns of feeding and drinking. NASA engineers are equipping the facility on Space Lab-3 with such devices (Schatte interview). The data collected will be the first of its type and will be very important.

7. Lighting. For studies of basic behavioral processes, the animal's habitat should have a standard light-dark cycle (e.g., 12hr light/12hr dark, or 16hr light/8hr dark). For the analysis of special problems concerning "endogenous" biological rhythms, continuous light or continuous dark could be used, but these are quite drastic environmental perturbations to use without explicit reasons.

8. Acoustic cues. Noise can be stressful, so it should not be intense (e.g., 100 db) for prolonged periods. It is important to remember that the rat's range of peak acoustic sensitivity is between 22 and 40 kHz, which is "ultrasonic" to humans. Rats use vocalizations in this high frequency range for social communication, including vocalizations that help coordinate the timing of copulation. This range of acoustic activity should not be "jammed" by spurious noises in their environment.

9. Airflow. Olfactory communication is of paramount importance to rats and many other mammals. Many of the biologically-active cues are small, light, volatile molecules. Rates of airflow through the habitats, air exchanges, and filtering should be examined carefully to avoid undesired effects. As mentioned earlier, airflow through the weightless environment might also be used as a device to control the direction and collection of waste.

APPLICATIONS OF NEW CONCEPTS: HABITATS FOR RATS IN SPACE

Let's apply to the design of habitats for rats in space the concepts of Habitat S/V, graspable surfaces, and visibility. Consistent with the reproductive theme of this report, these habitats are designed to house groups of rats and to provide a physical environment that will support social and physical interactions, maximizing reproductive success.

We will go on to consider some additional concepts in design and again provide some exemplary models. Note that these models and drawings are provided as illustrations of spatial principles, not full function. I have not included details of waste removal, air flow, feeding and drinking situations, etc. Each habitat is, however, capable of accomodating such necessities, while preserving its functional design.

Figure 13 depicts a space habitat that I call The Nautilus. It is essentially a cylinder wrapped around a central area; within the cylinder is a helix, attached to the wall and traveling the length of each taurus. At the distal end is an enlarged area, useful for social aggregations or for group feeding. It was designed with the following dimensions: the inside diameter of the tubular portion is 10 cm. The helix is formed from a band 1 x 2 cm. The cylinder and the helix are constructed of a transparent material, such as plexiglass, to permit visibility.

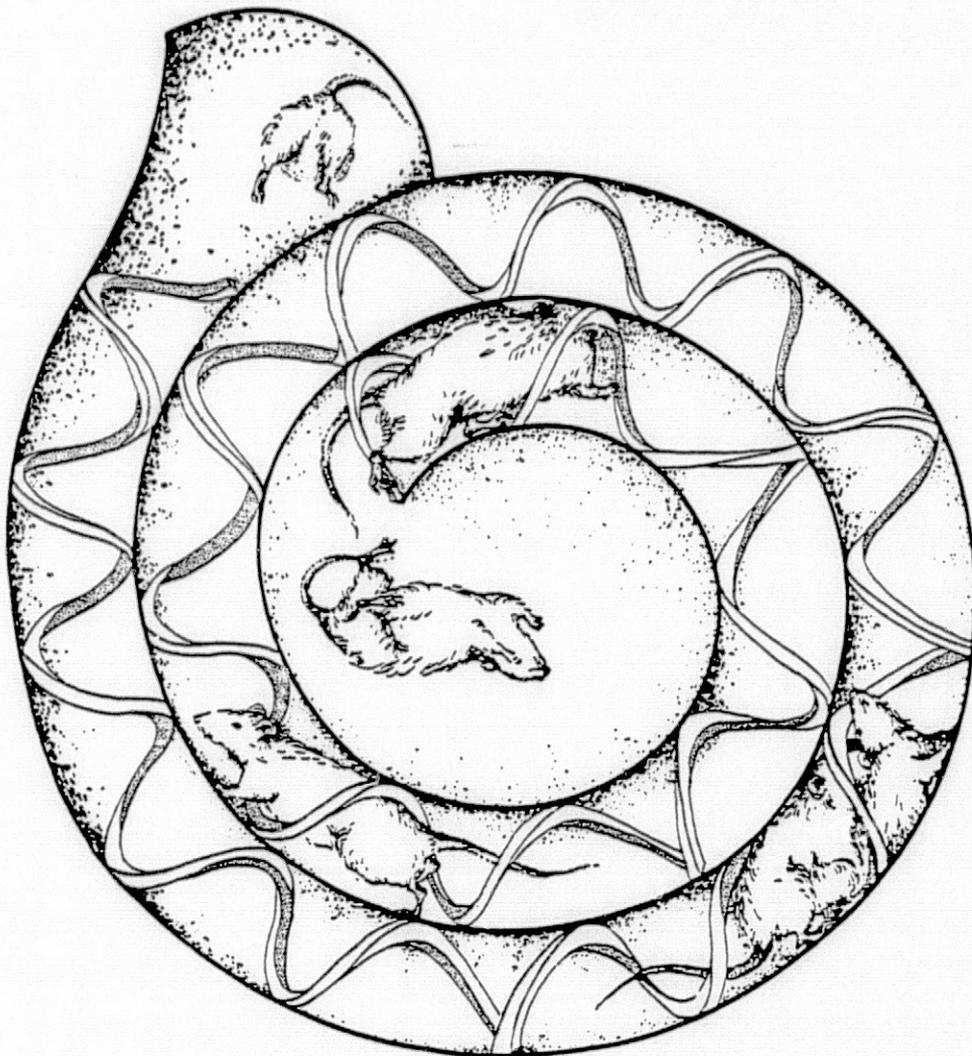


Figure 13. The Nautilus. The habitat is a cylinder wrapped around a central chamber, with a larger chamber at the distal end. A 1 x 2 cm helix, attached to the wall, runs the length of the cylinder. The periodicity of the helix provides a rat with a continuously graspable surface, yet is large enough to allow for two rats to interact.

One of the most notable features of this habitat is that the 10 cm diameter of the alley is large enough for rats to interact socially, as shown for example, by the mating pair at the 5 o'clock position. Yet, a single rat moving through the alley is, functionally, in a space that is only 6-8 cm in diameter. The periodicity of the helix is spaced so that an adult rat can always intersect one or two of the helix surfaces. We estimate the Habitat S/V of the Nautilus to be approximately .80, a remarkably favorable value.

One can imagine that in this habitat rats would adapt by developing a corkscrew trajectory, whereby they crawl or climb along the helix. Upon encountering another inhabitant, they would naturally adjust their positions to fill the space between the turns of the helix and, with the support of the surfaces around them, could enjoy normal, rat-like interactions of mutual sniffing, grooming, and other patterns of cutaneous contact. At each end of the alley there are areas for larger aggregations.

The shape of this habitat is also favorable for observation, particularly through a single lens. The nautilus shape and helix provide a large area in a flattened form that subtends a minimal area. Thus, depth of field is not a problem for a camera.

Figure 14 shows a disc-shaped alternative, called The Oreo. The disc is only 7 cm high, so Habitat S/V is large and can be enhanced by adjusting the interior design. Depth of field and width of field both favor visibility. Unlike the Nautilus, this habitat permits rats more directions of movement. The perimeter of the habitat is much like an alley. The internal space of the Oreo has a unique feature, intended to give the rats tactile contact, guidance, and graspable features for locomotion. The inside area contained a circle of small-diameter columns, arranged in a ring for this drawing, that can be placed to form alleys and passages, as well as support features. Use of these columns or pillars may help manage air flow and atmospheric exchanges in addition to improving the graspable S/V index of the habitat.

RATS AS DESIGN ENGINEERS OF SPACE HABITATS

An additional, important source of information and inspiration for new concepts in cage design are the rats themselves. I suggest that early space station research be planned to include tests in which rats can create their own burrows or dens by modifying the structure provided. This can be accomplished by filling one area of their chamber with a material such as dense polystyrene, into which they can gnaw and dig. During such an experiment, single rats, or groups of rats, will dig into this material, if their terrestrial behavior applies. On earth, they would create burrows and dens. The products of their behavior at null gravity might provide important and "creative" insights into features of cage design that would be engineered to the adaptive benefit of the rats themselves. Such an experiment would be relatively easy to conduct and, in addition to its pragmatic value, it would reflect to the public a basic interest and commitment of NASA to the concept of animal welfare in the U.S. space program. In this way, rats would be used as contributing "design engineers." It would probably be desirable to give rats the opportunity to burrow in space after they have been exposed to null gravity long enough to have adapted to the conditions.

If rats at null gravity create environments that resemble their

habitats on earth, we would have rat-made, or at least rat-designed, "subterranean burrows" in space. The bottom line in these plans and considerations must be the animal's well-being -- i.e., their ability to adapt to the environments under space conditions and to exhibit behavioral and physiological functions that reflect overall well-being. I shall discuss criteria for such judgements in a later section.

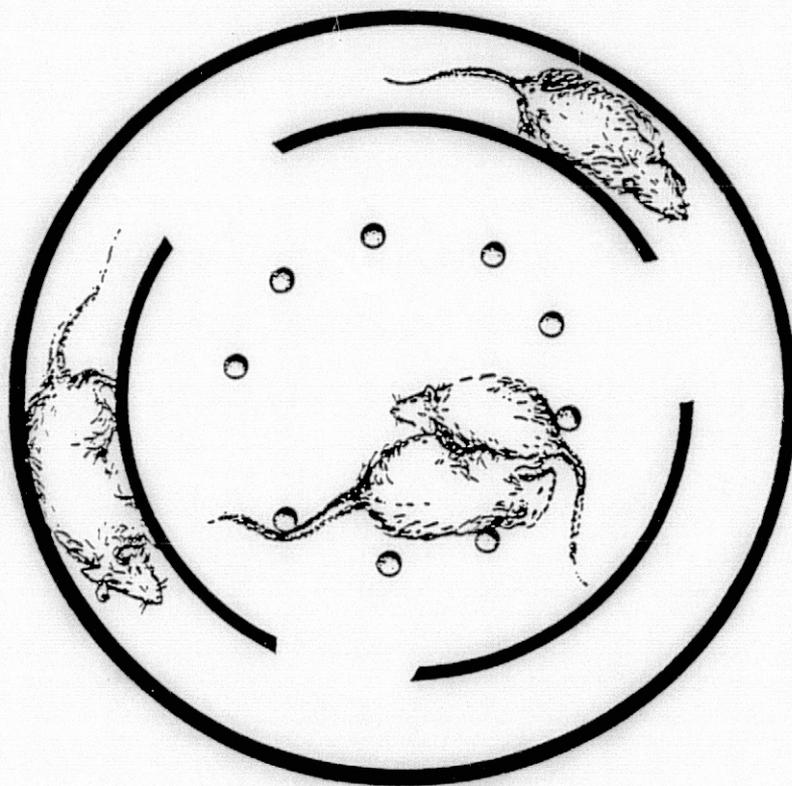


Figure 14. The Oreo. This disc-like form has an outer alley. The internal area is provided with numerous columns, shown here from the top, which can be grasped or treated as a supportive wall.

I have designed a sample habitat that show how we could put rats to work as design engineers and simultaneously glean other useful information from them. In an early sketch, done on a hot day, the habitat was reminiscent of a cool desert and was named The Neopolitan (Figure 15). This habitat is arranged within a parallel piped. It is trisected. One region is divided into man-made alleys that give rats tactile support and a region of small S/V. As can be seen in the figure, the central area is open. Rats in this region

would tend to float. The third section is drawn as an area that has already been formed into a rat-made burrow system. The grid-like area at its entrance is for traction and grasping during digging.

One of the beauties of this type of arrangement from the point of view of a behaviorist is that it offers the opportunity to observe the same animals in different surroundings. Thus, we could learn a good deal about the kinds of postures, movements, and activities that the same animal will display in each surround, and how these are patterned when it is in a niche that provides ample surface contact versus one that does not. For these studies, various types of movement notation systems would be used, providing preliminary data pertinent to questions beyond reproductive behavior, such as possible relationships between habitat design and energy expenditure.

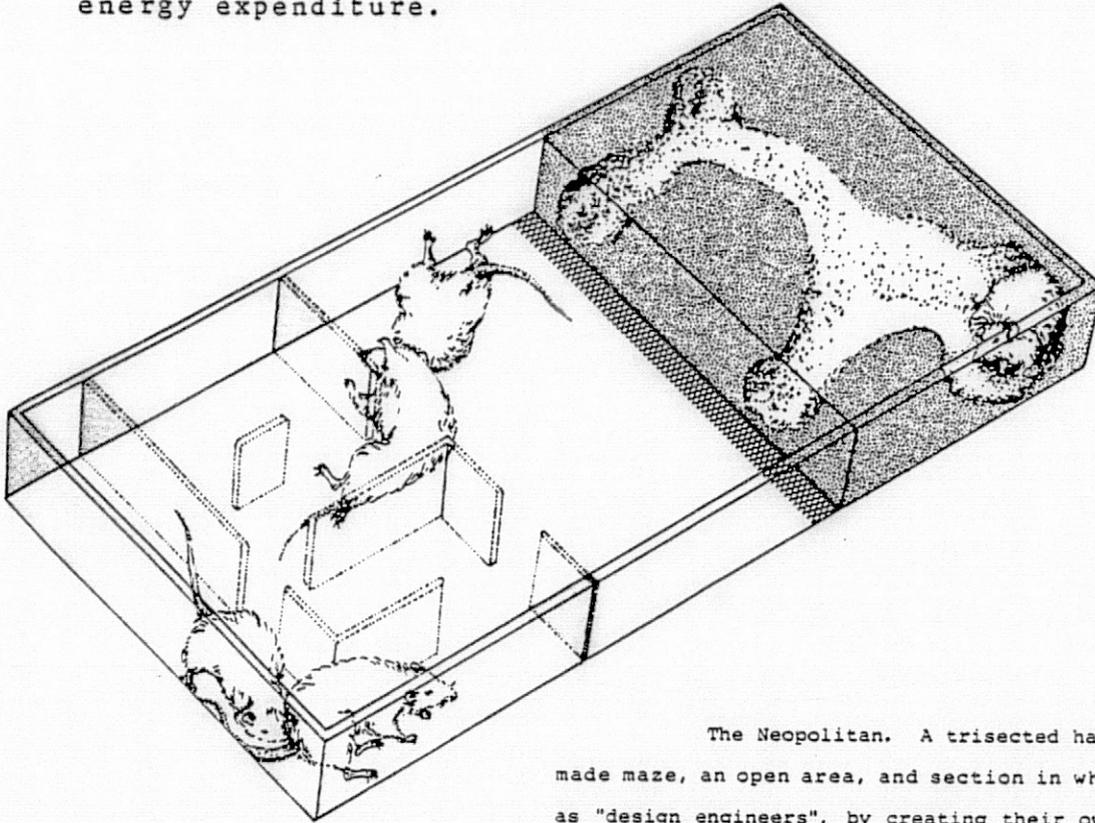


Figure 15.

The Neopolitan. A trisected habitat, offering a man-made maze, an open area, and section in which rats can function as "design engineers", by creating their own burrows and dens in space. This may provide us with important insights for habitats in space.

Preference tests. Another concept that I strongly recommend for inclusion into space station plans that involve animal maintenance is the use of preference tests. This incorporates the notion of the rat as an active participant in habitat evaluation. In this context, preference is an empirical term, referring to objective, measurable, behavioral choices of the animal. Think of these measures as a means of "conversing" with rats. We can ask questions such as, "When you are weightless, would you rather float around freely or use your limbs to control direction and speed?" and "How

do you like chambers with warm, smooth wall compared to graspable surfaces that are not solid?" Preference measures permit us to ask such questions and have the rats answer them. Preference measures are a powerful tool in behavioral research with complex, non-verbal organisms.

Measures of preference are derived from the kinds of visual records that I have stressed as priority items for all future animal studies. By designing experimental habitats that can be manipulated to offer the animals different types of surroundings, we can allow rats to sample each niche and tell us about their preferences by the distribution of their behavior. Figure 16 is a drawing of a type of Modular Habitat, shown here in partial disassembly. The model in Figure 16 would be set up as a three-way preference test. The niches that would be evaluated here would be: (a) The helix-filled field, designed to give rats a tunnel-like form yet free access to open areas and the ability to enter other helices or interact with passing rats. This is a modified application of the helix concept introduced above with The Nautilus. (b) The "bubble," a clear and voluminous area that would give the inhabitants the opportunity to move freely in three dimensions during weightlessness. I have tacitly assumed in the other designs that this is not optimal for the rats when, in fact, this is an empirical question -- that they can answer, and (c) a tabular tunnel system, providing maximal surface contact and physical support.

I include this module to emphasize that we must avoid making assumptions about the preferences of our experimental animals in space. By definition, these are habitats for a novel and unique environment, one which we do not yet understand and for which we cannot have predictive powers. We should be certain to allow the animals to show us novel and non-terrestrial patterns and preferences in these novel and non-terrestrial habitats.

The tubular portion of the Modular Habitat is removable and can be replaced with other modules for additional testing and comparisons of different niches in various combinations. Preference would be measured in terms of allocation of time relative to expected values if the animals were to distribute their time throughout the habitat at random. Deviations from the random probabilities suggest preference. Such measures would be pursued further by analyses of the nature and organization of behavior within each type of module (see discussion of The Neopolitan, which is another habitat amenable to modular manipulation).

Among the exciting opportunities offered by a space station is the ability for researchers to be present for the duration of observational studies, such as those described here, and therefore be able to choose preference test combinations on the basis of the animal's behavior. There are both practical and statistical advantages to studying the preferences of the same animals in different testing situations. Moreover, a space station can be used

for long-term studies, allowing us to analyse the behavior of adapted animals rather than adapting subjects, which would be more likely on a typical U.S. flight, which is designed for relatively short missions.

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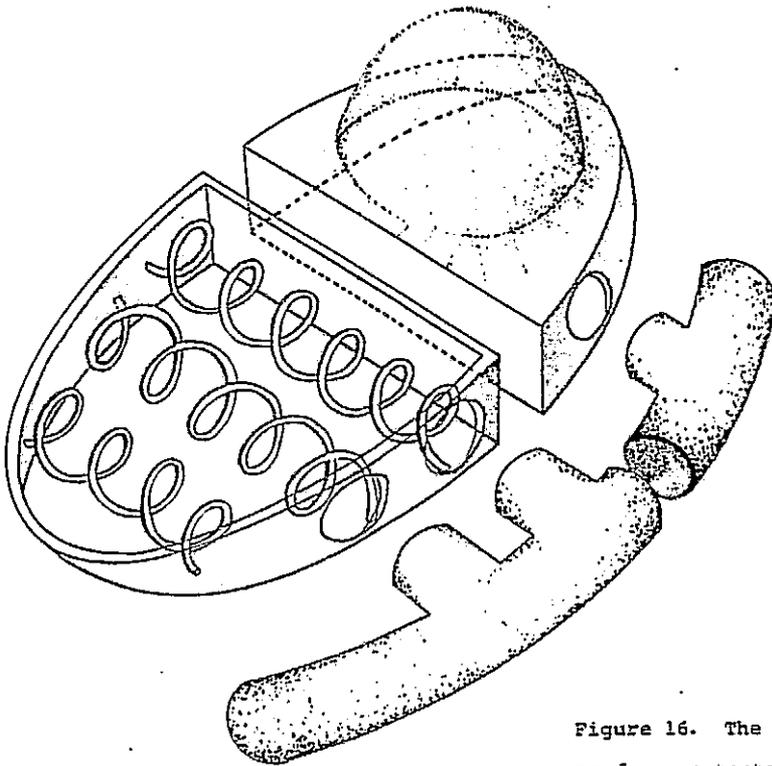


Figure 16. The Modular Habitat offers the opportunity to conduct preference tests with different combinations of habitat features. In this manner the rats can tell us about the kinds of habitats they prefer.

On-board Experiments with Gravitational Effects. The singlemost exciting and mysterious variable to study in space is null gravity. Most of the considerations reflected in this report relate directly or indirectly to the effects of weightlessness on biological systems. For purposes of such questions, it will be desirable to have centrifuge facilities on a space station to conduct on-board experimentation in which all temporal variables are matched as well as other space-related conditions other than gravitational forces. In the case of the present animal studies, we would repeat our observations in identical habitats that would be centrifugated to 1-gravity.

Figure 17 depicts a habitat design which is acceptable according to standards of S/V and visibility and which offers special advantages for studies involving centrifuged control conditions. It is a kind of Peristaltic Ring design, which capitalizes on the

burrow-den configurations and alternating zones of varying inside diameters, providing rats with tighter, tunnel-like portions as well as room for social contact (including copulation, as shown here). The bands, located at various compressed areas along the rings, depict connection points. This is also a modular unit, intended for preference tests, with interchangeable modules.

The most significant feature of the Ring, however, is that it is designed specifically to be amenable to centrifugation. In fact, the tripod-like stand, shown in the bottom of the figure, could be coupled to an axle and the habitat would "turn" into live-in centrifuge. Thus, the space station would carry both a null-gravity and a 1-g module of the habitat. On-board comparisons, featuring the 1-g control condition would be obtained.

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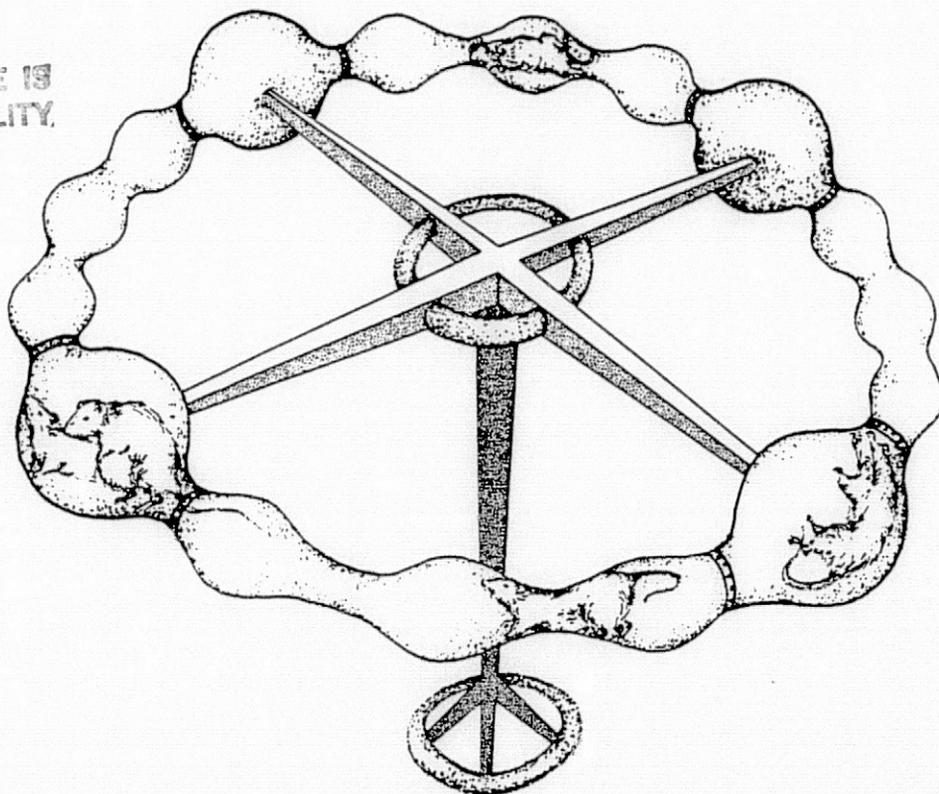


Figure 17. The Peristaltic Ring. This habitat is a tunnel with sections of expanded diameter. Note that it is designed with detachable sections so that, like the Modular Habitat, different types of components can be inserted for testing. This habitat can be mounted, as shown, to function as a live-in centrifuge, providing an ideal control condition with gravity-like forces.

ASTORRATS: SELECTION AND PREFLIGHT TRAINING OF ANIMALS FOR A SPACE STATION

NASA spends considerable time and money on programs to screen, select, and train human astronauts. Prior to traveling into space, each NASA astronaut receives as much exposure as possible to acquaint him or her with the kinds of experiences and contingencies that might be encountered during an actual flight.

There is no comparable effort made to select or prepare animals for flight. Why does NASA use such dramatically different protocols for selecting and training its astronauts than for its other mammals that may become space travelers? Obviously, NASA should and does make its primary commitment to the safety of its human astronauts. Nevertheless, it is unreasonable to forego application of the same principles that have produced an exemplary program of human space travel when we turn to the use of non-human animals for the same purposes. If anything, successful animal flights might require somewhat more preflight regimes since the animals, in contrast to their human counterparts, have not actively sought their role as pioneers in space, nor can they receive verbal instructions to help them adapt.

I recommend that NASA evaluate its procedures for preflight training of astronauts and the considerations that led to their development. Now is the time to apply such wisdom to the next generation of protocols: those designed for non-human animals.

There are at least two important reasons for undertaking the task of designing preflight regimes for animals. One concerns the pragmatics of successful scientific efforts. I have emphasized throughout this report that stress is one of the variables that must be monitored throughout all stages of animal research on reproduction and development. The goal of these measures is to identify stressful situations and act, usually in anticipation, to eliminate or reduce the occurrence of stress or discomfort to the animals. A major goal of this research program is to create a comfortable and safe habitat for animals in a space station, one in which they will procreate and live in a state of well-being. From a scientific standpoint, we know that excessive levels of stress are detrimental to biological rhythms, interrupt the expression of adaptive patterns of social and sexual behavior, and perturb the rate and form of growth and development.

A second reason for conscientious plans for a specialized program of preflight training concerns the range of animal welfare issues on the horizon for NASA and other agencies in the public eye. Groups that are dedicated to monitoring the standards in research facilities may look critically at a prominent program such as NASA's and take the opportunity to publicize their cause by criticizing poor planning or outcomes of animals in space research. Moreover, some of their challenges might deserve to be heeded.

because both the animals and the research can benefit from adherence to such suggestions.

I suggest that NASA can demonstrate admirable prescience by taking the initiative and establishing a mini-program for selecting and then training cohorts of "astrorats." It should be emphasized that this is not merely a public relations effort. It makes good sense -- scientifically, economically, and ethically. The program may be modelled after that used for humans, but it should be aimed foremost at selecting and preparing animals for smooth adaptation to space.

The behaviorally-oriented program of reproduction and development discussed here is an ideal format for such efforts by NASA. Most of the research is non-invasive and is based on establishing adaptive behavior in a space station. It is the kind of effort from which many citizens and taxpayers would derive enjoyment, intellectual stimulation, and aesthetic pleasure. In addition, society will benefit from seeing that it can simultaneously support humane treatment of animals and good scientific efforts.

I believe that NASA is charged with numerous roles in our society and that these roles will expand in the coming decades, particularly with the establishment of a permanent space station. This is an opportune time for NASA to set a public -- if not international -- standard for its pioneering work with non-human animals in space. Such a program can be established in a way that is both ethically, scientifically, and culturally defensible. In doing so, all citizens, the animals, and science can enjoy mutual benefits.

The design of a preflight program probably requires an interdisciplinary study group, combining experts from human and animal research. At various stages, it would be best to include NASA scientists and engineers, astronauts, veterinarians, research scientists, specialists in animal husbandry and zoo management, as well as representatives of responsible animal welfare groups.

I have prepared a set of examples of experimental protocols to prepare animals for space flight. The goal is to attenuate the stress that rats might experience when they initially experience null gravity and to shorten the period required for adaptation to space conditions. The strategy has been to design ground-based experiences that might provide some generalization to space conditions or offer the animals some degree of experience with novel environmental conditions to which they can adjust.

1. Neutral bouyancy experiences can be provided by introducing the rats to comfortable aquatic environments and allowing them to interact with other animals under such conditions. (Rats are known to swim and even to dive for food under natural conditions; they

take to water quite well.) These experiences should be introduced gradually by beginning with only a shallow layer of water and gradually increasing the depths of water to which they are exposed until they are interacting with other rats while experiencing the buoyancy provided by the medium.

2. Rats should be housed in habitats like those to be used on the space station. Their movements, social patterns, feeding behavior, circadian rhythms, and stress indices should be monitored carefully. The purpose here would be to habituate or familiarize the rats to the habitats in which they would live at null gravity, so that extra novelty is not added to the zero-gravity experience. Similar efforts for habituation should be made for diet, food stations, and the co-inhabitants of the cages.

3. Rats should be subjected to a gradually increasing exposure to vibration and rotation. They should be given a gentle program of desensitization to environmental perturbation. The reality of these manipulations can be monitored with stress and automatic nervous system indices.

4. Individual differences should be studied carefully to identify animals that adjust readily to space-related stressors. Hyper-reactive animals should be screened and not used. Breeding studies might be tried to ascertain whether a line of space adaptable animals can be created.

5. Animals should be given repeated experience with adapting to hyper-gravity forces via centrifugation and then experience with stepping down to 1-g. Dr. Jiro Oyama (NASA-ARC) has spent many years studying adaptation to hyper-g forces and agrees that this might be a very useful procedure for preflight training, giving the animals experience with decreased gravitational forces.

It should be remembered that humans report that the experience of null gravity is quite pleasant. Stress endured by animals at null gravity is probably related mostly to the novelty of the situation. For rats, and most animals, environmental novelty is stressful. The program of preflight training would be designed primarily to reduce the novelty effects on the rats. The examples given are a sample of possibilities. A corollary to these efforts will be to combine these manipulations with systematic studies of stress and adaptation. It is imperative that these studies and their evaluation be designed carefully so that NASA's efforts to plan a sound program of preflight training for experimental animals can be presented intelligently and reasonably to both supportive and potentially critical audiences.

STUDIES OF REPRODUCTION FOR A SPACE STATION

Basic experimental groups. I recommend a conscientious effort to examine a select group of behavioral and physiological parameters for space station studies, first measuring each on the ground, then at frequent, continuous intervals in space, and again -- postflight, on earth. There will be four different groups of animals. (1) Foremost will be the Flighted group, maintained in the Space Station under conditions of Null gravity. (2) Second will be an On-board, Centrifuged Control group. These will also reside in the space station, but they will be maintained at 1-g, by virtue of an on-board centrifuge. On the ground will be two additional control groups: (3) It would also be desirable to have a Simulated Flight control group, residing on the ground. These animals would receive accelerative forces, vibration, noise, temperature, and impact stimuli yoked to the Flighted group, but their mock satellite would never leave earth. (4) The so-called Synchronous controls will be used as a baseline, representing the "unmanipulated," normative group. These would be animals simply maintained under standard, ground-based laboratory conditions and will be referred to throughout this report as the Vivarium Controls.

STAGE I: SPECIFIC MEASURES TO INCLUDE IN STUDIES OF PRECONDITIONS FOR MATING

1. Stress. It will be essential to pay careful attention to measures of "stress" in rats used for these mating studies. I interviewed Dr. Seymour Levine of the Stanford Medical School (Appendix E), one of the country's experts on stress in animals and, in particular, on the complicated effects of stress during pregnancy and lactation. There are available both urinary and plasma corticoids that can be used as measure of stress responses. Dr. Levine discussed some of the vicissitudes of this work, but without question it is of central importance to the design and evaluation of space habitats.

Moreover, it is known from decades of ground-based research that various forms of stress, particularly during pregnancy, can have a profound and long-lasting effect on the offspring. Some of these effects can be attributed to chemical and hormonal aspects of the intrauterine environment. Other aspects are transmitted behaviorally, via parent-offspring interactions. Thus, although stressed rats can produce viable offspring, there are often undesirable residual effects that can be avoided by first monitoring stress levels in the adults and taking measures to reduce or eliminate their levels of stress.

It should also be noted that pregnancy can also bring with it a condition that "buffers" the pregnant animal from some of the

detrimental effects of stress. Such data could provide useful clues to reducing or controlling stress in other animals, including man.

Programs to monitor and to alleviate stress in flighted animals is a fine and important example of a case in which the goals of humane treatment of animal subjects and the interests of good science are mutual. It is in the interest of the animal's well-being that we optimize the conditions for them and reduce or eliminate unnecessary stress.

A space station and its personnel should be equipped to collect and store blood plasma and urine. This could be analyzed on Earth or, if radioimmunoassays can be performed on a space station, full research efforts could be conducted in space.

2. Feeding and Energy Balance. To some extent, these variables tend to co-vary with some, but not all, forms of stress response. Adequate, well-regulated levels of feeding and energy balance are usually signs of healthy animals. The energetic demands of mammalian reproduction, particularly for females, raises these measures to first level importance. Research protocols on previous flights have not included basic metabolic measures, much to the surprise and horror of many of the experts with whom I discussed these issues. It is clear to them that it is essential to both the welfare of the animals (and of humans) and to the hopes of doing organismic studies, that basic research in energy balance and metabolism be included in space station studies.

The techniques that are relevant here are careful measures of food and water intake, the ability to offer animals dietary choices for specific kinds of studies, apparatus to measure metabolic rates: oxygen consumption, CO₂ production, energy excreted, and body composition. Most of the assays can be performed on Earth, provided that suitable facilities for collection and storage of samples are available.

3. Estrous cycles. I have mentioned that environmental or physiological perturbations can disrupt estrous cycles. Indeed, one of our hypotheses is that the female rats in the Cosmos experiment became acyclic as a result of their habitat. Space station research aimed at reproduction must include regular studies of estrous cycles in the female rats. The procedure is quick and requires only a light microscope with which to examine a small quantity of vaginal wash.

4. Spermatogenesis. Sperm production by male rats should be quantified as a function of exposure to space conditions. Similarly, it would be desirable to evaluate the motility and fertility potential of the sperm themselves. This requires basic microscopic methods.

5. Sensory function. The coordinated responses made to each

other by male and female rats throughout the entire reproductive cycle requires that each participant perceive and respond to a variety of olfactory, visual, auditory, tactile, vestibular, and proprioceptive cues. Elimination of some cues, such as olfactory stimuli, can have a disastrous effect. It is therefore important to protect sensory function and insure that space conditions do not exert detrimental effects on sensory coding and perception. Fortunately, a variety of methods are available to quantitatively evaluate sensory function in animals, including rats. It is beyond the scope of the present report to review these methods in critical detail, but it should be emphasized that such methods are readily available on Earth and could be adapted to a space laboratory.

Available methods vary in sophistication, precision, and the degree to which prior training or special treatment of the organism is needed. The most applicable methods for diagnostic use in a space station would be tests that rely on so-called unconditioned, or reflexive, responses. This way, prior training is not needed and the experimenter does not have to be concerned that the effects of the training have waned, or that the animal is failing to perform learned reactions for some other, secondary reason. Unconditioned reactions can be used to assess olfactory, visual, auditory, and other sensory functions. The apparatus required for such tests are simple and there is no necessity to restrain, inject, or stress the organism in order to make the tests. Experimenters in a space station could interpret the results of such tests and then adjust experimental plans accordingly.

6. Motivational processes. I have included this category to represent the array of organismic conditions that can affect the expression of other behavioral goals and processes. Sexual motivation is, of course, crucial to animals, but its expression can be displaced by other motivational conditions. Extreme hunger is one example. Another relevant possibility is that animals that are not adapted to space conditions might experience some form of malaise akin to that associated with motion sickness. (Rats, by the way, cannot vomit because of a sphincter in their esophagus. They can, however, demonstrate evidence of vestibular-associated malaise.) We can assess the animals sexual motivations in a number of ways. We can get a quantifiable measure of sexual motivation by presenting odors (e.g., the urine) of other rats that vary according to the reproductive state. The amount and persistence of sniffing investigations can be used to gauge the degree of sexual interest that is generated.

7. Ground-based studies. Although I have stated that numerous methodologies are available, it would be advisable for NASA to investigate these systematically, by conducting a critical review and survey of these methods. An important aspect of this review would be to examine the interpretive boundaries of each type of test. Some tests reveal whether the organism detects a signal, but it cannot be determined whether the animal can tell the difference,

say, between two different sounds that vary in pitch. A rat might be able to detect odors but not discriminate between the odors of an estrous versus an anestrous female. Some tests might be excellent in terrestrial applications but would not work well at null gravity because the animal's response is gravity-dependent. Finally, such a careful review is important for considering the size and weight requirements of each alternative, for these might affect selection and dictate the need for further refinement.

It should also be noted that one type of sensory testing that probably deserves some extended consideration is examination of vestibular function. This is an area for which there is a respectable database for rats and other laboratory animals, but this sensory system has been terribly unrepresented relative to other sensory modalities. On the basis of my experiences with the interviews conducted for this report, however, I am sure that this situation could be rectified. It would be very useful to pursue a set of vestibular tests that could be used at null gravity and in an on-board centrifuge. NASA's most efficient route to obtaining good vestibular tests would probably be to contract some answers to specific functional questions rather than to wait for vestibular studies to catch up and provide such information in the course of its overall development.

STAGE II: STUDIES OF COURTSHIP AND COPULATION

We have discussed factors relevant to caging and/or habitats for rats in a space station. These factors were discussed in the context of "preconditions" for mating, but it is appropriate to reiterate these factors at other stages consideration. It can be imagined that some habitats could appear non-stressful because the rats eat and ambulate, yet they may fail to mate. Such a result could be caused by missing features of the environment, features needed for some mechanical aspect of copulation, for example.

Behavioral processes. Figure 18 shows a solution to special needs of particular behavioral activities in space. The device illustrated in this figure of mating is an "elastic sock" which is designed as a potential add-on unit to any of the habitats that were presented in the earlier section of this report. It is designed to provide rats with additional sources of tactile stimulation, enhance proprioceptive cues from movements, and increase the forces that can help "lock" the male and female during copulation at null gravity. Rats enter the sock through tunnel-like openings during courtship movements, and remain within it voluntarily during copulation.

The single most important technical aspect of studies of courtship and copulation in space will be visual data. The experiences of the Cosmos-1129 flight, and the large number of plans that hinge upon a detailed familiarity with movement, posture, and

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locomotor activity by animals at null gravity all point to the collection of visual information as a priority of this research. The same four experimental groups of animals would be pertinent to all studies of courtship and copulation.

The amount and quality of courtship and copulation should be studied during the process of adaptation to null gravity. Copulatory activity in particular will warrant close scrutiny in order to evaluate crucial elements of the interactions, such as the male's ejaculatory thrust, and the females' ability to maintain lordosis appropriately to facilitate the formation of a good baginal plug. Direct examinations by an on-board experimenter would be important at these stages.

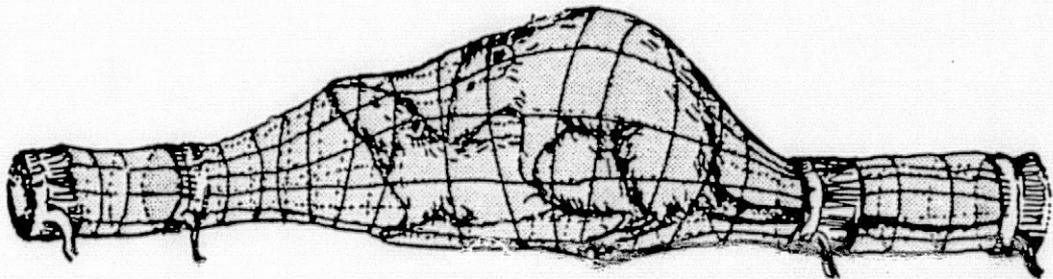


Figure 18. The "Copulatory Sock".

This device is designed to provide rats with mechanical support to facilitate copulation at null gravity. It is an elastic tunnel that the rats voluntarily enter, which expands with their bodies and provides light compression to prevent the male's thrusting from pushing him off the female's body.

The conduct and success of group mating should be a major concern for research on reproduction by rats in space. We will be interested in whether rats display signs of coordinated group mating in a null gravity environment. What forms will the group mating process take? Will there be evidence of reproductive enhancement from the group processes? Will differences in group mating patterns correspond to changes in the reproductive physiology of individuals?

In addition to obtaining answers to these kinds of questions, the basic findings from these observations will, I believe, be potentially illuminating to students of behavioral adaptation.

Never in the evolution of terrestrial vertebrates has it been possible to test the limits of adaptation so rigorously and dramatically. The responses of the rats, regardless of the immediate outcome, will be our first glimpse at an unknown aspect of both individual and group adaptation. Thus, the results of these studies are bound to be seminal and will stimulate the imaginations of many scientists and laymen alike.

Of particular interest will be the basic information on the reproductive success and overall performance of animals in different flight habitats and of the same animals in the habitats that offer different sections of varying characteristics. Again, of interest here will be: (1) rates of adaptation and the expression of reproductive activities of rats housed in different kinds of environments (single option habitats), (2) behavioral preferences and consequent rates of adaptation displayed by rats housed in environments that offer choices of niche (preference measure habitats), and (3) comparisons of behavioral topography and outcomes of rats in the same immediate environments when these are their sole options versus situations when the animal can willingly enter and leave a particular type of surrounding.

We shall want to know if preflight experiences and training have facilitatory effects on the onset of normal sociosexual behavior in space. Again, this is an issue of adaptation, using a meaningful behavior as the major index. The results are thus relevant both to the immediate question of reproduction as well as to a broader appreciation to the general process issues of stress and adaptation. Another aspect of adaptation that could be studied in this regard would be the courtship and copulatory behavior of animals that have been adapted to space and then returned to Earth. This is a question of readaptation and might be helpful in analyzing the process. An important dimension of such investigations will be to measure "savings" accrued by experience with adaptation. Will animals adapt to space more readily upon second and third exposure? Will readaptation be different with practice? If so, we can seek to separate residual physiological changes from rates of adaptation. This could be an important dissociation to make in early studies for a poorly understood process.

Contingencies. We must anticipate the possibility that rats will not court and copulate, no matter how thoughtful and well-intentioned our initial "best efforts." Two tracks should be followed in the event of reproductive failure, or impairment. First, we will have to begin the systematic analysis of the dyadic system: identify points of "decoupling" and determine the nature of the breakdown. There are literally dozens of possibilities that can be imagined. To review these now is not appropriate, but it would be important for NASA to make itself and its program planners aware of the possibilities and be prepared to intervene with batteries of behavioral and endocrine tests to diagnose and respond to the situation.

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The second track should be pursued simultaneously. This would be the incorporation of a nuptial centrifuge into the space station. Figure 19 depicts such a device. As shown here, it is two mating compartments mounted on a centrifuge device that could provide the g-force to support the mating behavior of rats in a space station, if they would otherwise be unable to copulate. This chamber should be equipped with observational equipment to determine the rate and kind of adjustments that affect mating.

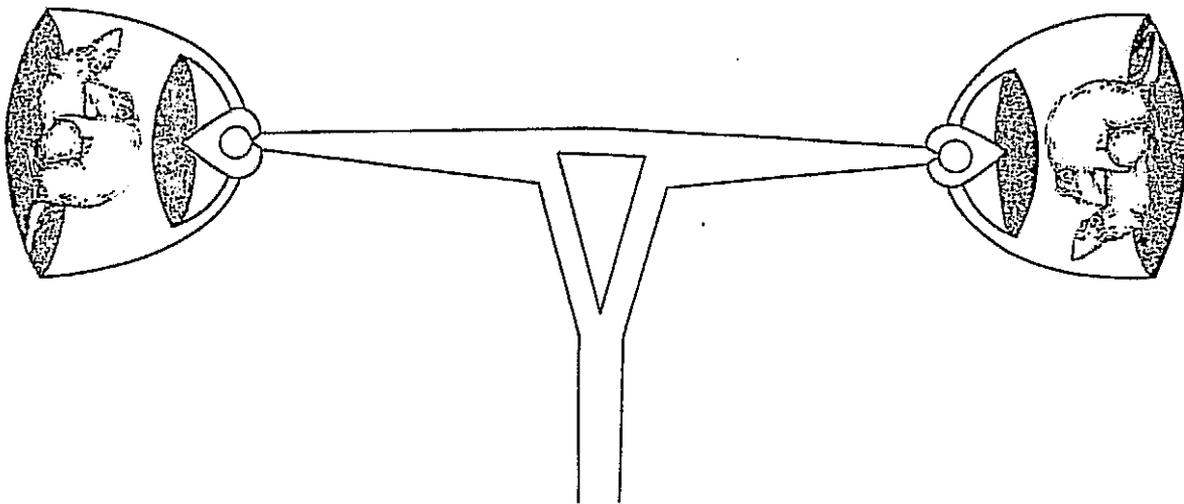


Figure 19. The Nuptial Centrifuge.

This contingency device is an on-board centrifuge with mating compartments, used to provide rats with gravity-like forces to support mating, before they are returned to their normal, null-gravity habitat on the space station.

Reproductive failure might not be analyzed completely by observational methods alone. It will be advisable, again, to prepare for urinary and blood assays of hormones and metabolites to correlate with behavioral data.

One of the most exciting prospects are the results of analyses performed on animals born and reared in space. Unlike any other organisms ever tested, the first-derived generations born on a space station that will be the first "children of space," organisms in whom we might see embodied the results of epigenesis of terrestrially-selected genes acting through development in an "unexpected" environment. Here, most of all, we may see the limits of adaptation.

Ground-based Research. The initial few generations of questions and studies can be chosen well in advance of the actual launching or creation of the space station. Due to these unique kinds of research that the space station will offer, it is unlikely that some of the questions could be answered in other ways.

I would recommend that NASA establish a ground-based program of study of mating behavior. This would be conducted under simulated conditions using selected, "flight-trained" rats. Movement notation systems would be used to measure group dynamics in these habitats. Such work has never been done, despite extensive biobehavioral studies of sexual behavior in rodents. This is an essential preface to the space experiments. It is advisable to start well in advance. An early start would also give NASA and the biobehavioral community a chance to learn about each other -- a valid aspect of the development of optimal working arrangements.

NASA should therefore establish its own space-relevant database of rodent reproductive behavior. This can be created quite efficiently, because of available knowledge.

I also recommend some experimental exploration of ground-based studies of mating in simulated space-like conditions. What might these be? One intriguing possibility would be to study mating behavior on earth in animals with vestibular dysfunctions. To my knowledge, this has not been done. In the past few years, various ototoxic drugs have been identified. These drugs disrupt the peripheral vestibular apparatus, particularly the crista, macula, and types I and II sensory hair cells. Recently, the drug sodium arsenite (atoxyl) has been shown to be specifically ototoxic in rats. New surgical techniques for labyrinthectomy have also been perfected. It is also possible to identify inbred mutants that have natural otolith disorders.

I therefore recommend that a modest program be established to learn how to study sexual behavior in vestibular-disrupted rodents. I believe that there will be a valuable transfer of skills from such ground-based experiences to the situations encountered in space.

Thus, once the initial questions are chosen, ground-based correlaries can begin in anticipation of the actual space studies. This strategy would offer the valuable opportunity to test and refine all measures and procedures so that the experiments in space can be chosen efficiently and run quickly.

STAGE III: STUDIES OF PREGNANCY IN A SPACE STATION

The desirable studies of the course of mammalian pregnancy should be structured in a parallel manner to those of the preconditions as well as the courtship and copulation studies. The

same experimental and control groups would be used. The course of pregnancy in space would be studied, hopefully with animals inseminated in space, either naturally or artificially. These pregnancies would be compared to those arising from terrestrial inseminations but flown thereafter and, of course, with terrestrial pregnancies that never leave the ground.--

1. Body size during pregnancy. A useful index of the trajectory of pregnancy is the increases in the dam's body weight. In null gravity, we can rely on changes in size (e.g., girth); these parameters should be studied on earth to obtain reliable normative data.

2. Energy balance during pregnancy. Changes in energy balance are associated with increased consumption of food and water that is put both into the growing fetuses and into energy stores in the dam that are used when lactation begins. Although much research has been performed on the energetics of pregnancy and lactation in rats, it may be economical for NASA to conduct a program of energetics research on earth, designed to identify the best diets, methods of presentation, and measures to be made. One issue that warrents attention is whether to offer pregnant dams a standard, "balanced" diet in which all nutrients, minerals, etc. are contained in predetermined proportions, or alternatively, to develop a "cafeteria" diet whereby rats ingest fats, carbohydrates, proteins, salts, and other nutrient items in combinations that they self-select. The self-selection, cafeteria approach could be valuable means by which rats in space could tell us about particular dietary needs or strategies that are peculiar to metabolism at null gravity. The demands of pregnancy and lactation are additional factors that could easily interact with the space variables in ways that could be best understood with the direct help of rats themselves. Curt Richter, the great psychobiologist of this century, championed the use of such cafeteria tests.

3. Self-grooming. Normally, the pregnant rat show a distinct change in her self-grooming during late pregnancy. She directs increased amounts of licking and associated paw movements along her "nipple lines," the body area running down the two rows of six nipples each, and around her anogenital region. Pregnant rats fitted with disc-like collars that prevented self-licking of the nipples display a 40% reduction in mammary development and produce insufficient amounts of milk. Control rats wearing similar collars, but notched to permit self-grooming, had normal mammary development and produced plenty of milk.

This simple but dramatic observation is a good example of the interpretive power offered by well-chosen behavioral studies. It is easy to imagine a carefully-executed flight experiment indicating that nursing infants cannot grow normally in space or after their mothers have been at null gravity during pregnancy when, in fact, the causal explanation lies in a disruption of self-grooming during

pregnancy. Behavioral observations could help avoid such a mistaken conclusion. Even better, such behavioral concerns could be used to design and test flight habitats in which animals will display their normal behavioral repertoire and preserve subtle, but important, features.

4. Physiological aspects of pregnancy. There are two aspects of physiology relevant to our concerns: changes in the female herself, and the translation of these changes as they affect the offspring within her. By definition, both aspects are inextricable, but we will reserve discussion of offspring effects for the section on development.

The upcoming Cosmos 1983 flight will carry the first pregnant female mammals into space. Their fate will help identify important physiological variables for study. At this early point in our considerations, it is perhaps most important to reiterate that space biology, as performed by both U.S. and Russian scientists, has ignored study of female physiology.

Without baseline data on the non-reproductive female, our ability to anticipate the most important variable during pregnancy is hampered even further. Nevertheless, based on the small amount of space-relevant results with males, early space studies of pregnancy should be designed to examine: (1) the size and constituency of body fluid compartments, since pregnancy is accompanied by proportionate increases in extracellular fluids. Note that extracellular fluids have decreased in males during spaceflight. Will this mean the problem will be worse during pregnancy? Will the female's physiology protect her from these changes? (2) Calcium metabolism, also shown to be vulnerable to space conditions, is a prime topic for study. It is vital to female homeostasis during pregnancy as well as preparation for lactation and, of course, bone deposition in the fetus.

It is interesting to note that one of the peculiarities of pregnancy is often a change in calcium metabolism that leads to strengthening of bone. Perhaps the physiology of the pregnant rat in space will offer insights into some of the threatening problems posed by bone weakening that has been found in males exposed to space conditions.

The female's hormonal profile during pregnancy will, of course, require monitoring in space. My interview with Dr. Seymour Levine, a Stanford Medical School expert on hormonal aspects of stress during pregnancy, was most interesting (Appendix E). Dr. Levine remained equivocal on his predictions about hormonal changes during pregnancy in space, but he pointed out that the pregnant female is often "buffered" hormonally from stress effects that her non-pregnant counterpart might suffer. Issues of stress and other endocrine factors relevant to life in space appear throughout the report. In addition to enhanced caring for pregnant research

animals, a space station lab devoted to endocrine analyses could learn from pregnant animals about how other space denizens could be similarly buffered.

It will be of the utmost importance to monitor energy balance. Norway rats can regulate the rate at which they gestate a litter, partly on the basis of their overall energy balance. If space conditions produced a net deficit in the energy balance of a pregnant rat, she may adopt the "adaptive" strategy and decrease the rate at which she invests energy in her gestating fetuses. It would be an error to attribute reproductive delay to space conditions when it might reflect a primary adaptive response to a secondary deficit in energy availability.

Questions of mother-fetus fluid balance, hormonal milieu, and calcium metabolism are all major issues looming on the horizon. We will have no idea what we are to confront until the early, basic studies are run. It is the case, of course, that the 1983 Cosmos flight is designed to provide one set of data points pertinent to these issues. Pregnant rats will be flown for one week, beginning on Day 12 of gestation. The effects of space conditions on pregnancy will be evaluated by examining dams and fetuses immediately upon recovery as well as studying the flighted mothers and their space-gestated offspring on Earth, born two days after recovery (if the pregnancy remains on schedule).

During these pregnancies, in space and on Earth, each of the parametric combinations described earlier we will want to monitor. The self-grooming issue will be a significant one, as a means of better understanding postnatal outcomes. In addition, self-grooming and other forms of physical activity are likely to be experienced by the developing fetuses. Thus, their prenatal environments may be altered indirectly by the differences in stimulation related, for example, by space-induced differences in activity or overall muscle tonus during spaceflight. These hypotheses can be supported and/or refuted with carefully collected observational data in combination, perhaps, with electromyographic measures.

PART III

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PART III

MAMMALIAN DEVELOPMENT IN SPACE

An historical introduction often gives the reader perspective, but there is no history of research on mammalian reproduction in space. For many, this is a great disappointment. Developmental research is one of the most scientifically exciting arenas for space research, bringing with it unique and important experimental questions. (For examples, see interviews with Souza, Schatte, and others. In addition, many specialists not represented in these transcripts have also identified mammalian development as one of the richest areas for space study.)

NEURO-ONTOGENY EXPERIMENTS ON COSMOS '83

In October, the Soviet biosatellite Cosmos '83 will begin the history of space research on mammalian development. As a continuing part of Soviet hospitality, several U.S. investigators, working through NASA, have been invited to take part.

The satellite will include ten pregnant rats that will begin the one-week flight on Day 12 of their pregnancies. Five will be sacrificed at recovery for embryological studies. These studies will offer the closest glimpse to date of the effects of null gravity on body composition and prenatal, mammalian development.

The remaining five females will give birth. If space does not alter the length of pregnancy, the rats will give birth in Moscow about two days after recovery, or 21.5 days after fertilization. These newborns will be the first mammals to undergo gestation in space.

The author, J. Alberts of Star Enterprises, has designed and built a laboratory to test maternal behavior and postnatal development.

Postnatal studies. The postnatal studies are intended to provide a broad, quantitative assessment of infant growth and maturation. A battery of sensory and motor tests will be administered throughout the early postnatal period until weaning (Day 21). Functional assessments of vestibular, tactile, olfactory, auditory and visual senses will be made. Motor and reflex development will be tested with standardized measures. Several measures of individual and group behavior will be used to study behavioral development. All data will be recorded on video, magnetic or computer-compatible storage devices so that copies are also available for other scientists.

Assessment of Maternal Behavior. The behavior of the mothers, particularly in relation to the offspring, will be monitored and

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recorded using time-lapse video and computerized devices. The following will be measured: number and duration of nest bouts; time spent nursing; patterns of milk ejections; responsivity to pup vocalizations; nest temperature; food and water intake; and circadian rhythms.

POSTNATAL DEVELOPMENT OF THE RAT AND ITS APPLICATIONS TO
BIOBEHAVIORAL STUDIES IN SPACE

Like the adults, infant Norway rats are excellent subjects for research in biomedicine, behavioral biology, and other areas of the life sciences. Since the rat is born in virtually a fetal state but matures rapidly, it provides an excellent subject for developmental studies. Figure 20 summarizes some of the most stunning changes in the morphological, behavioral, and sensory development of the rat during its first three postnatal weeks. Let's briefly survey the developmental picture:

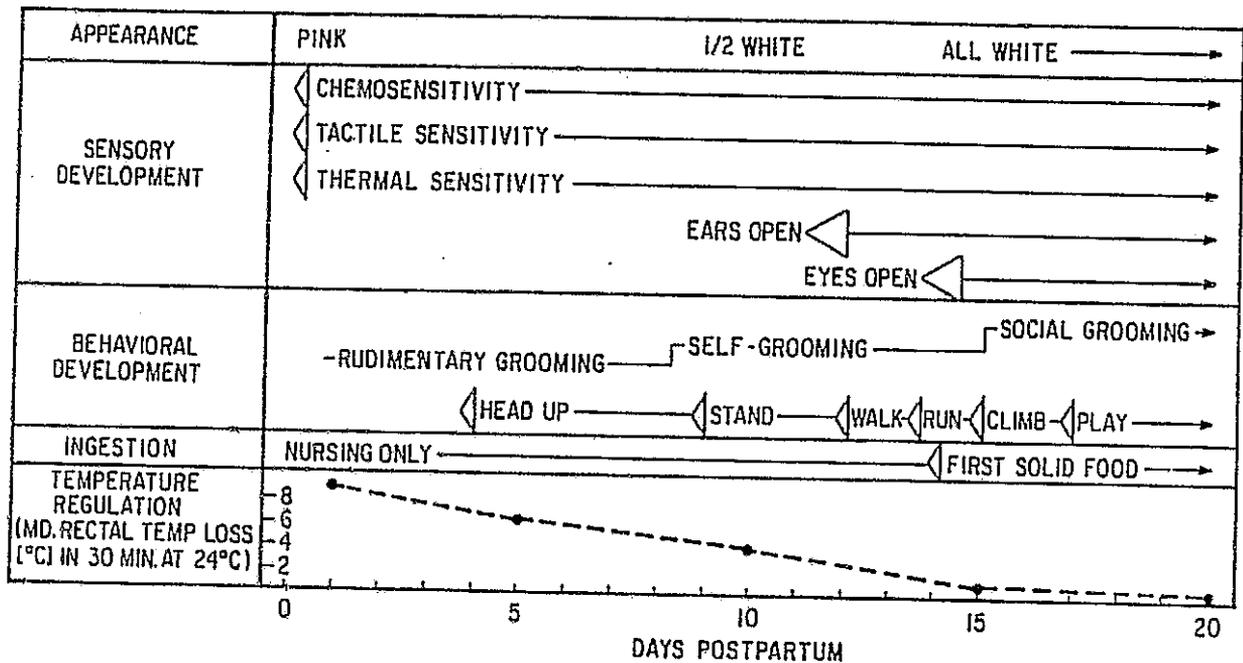


Figure 20. Summary of early postnatal development of the rat.

There are 8-10 pups per litter. They are born hairless, with transparent pink skin. Their eyes and ears remain sealed for about two weeks. Early in life, they can neither see nor crawl. In all terrestrial habitats, the pups initially live in a clump, or huddle, which is both a physiological and behavioral unit. Their ability to regulate body temperature and energy expenditure, for instance, is

dependent upon the huddle. In the absence of gravity, it may be difficult or impossible for pups to form and maintain this crucial aggregate. Unless the natal nest is equipped with special temperature control mechanisms, the effects of space may be disruptive, if not disastrous.

Besides huddling, one of the major behaviors of the infant rat is nursing. Pups spend many hours each day attached to the mother's nipples (she has 12), even though much of their sucking is non-nutritive.

It is difficult to imagine how the orientation reactions and movements required to find and attach to a nipple will be coordinated when pups and mother are in a null gravity habitat. We do not know how much mobility and control the pups will have or whether vital olfactory cues will be disrupted by peculiarities of airflow. The roles of vestibular cues have not been studied carefully, even though they are probably an important part of early behavior in many animals, including primates (see interview with Sackett).

SENSORY DEVELOPMENT

Different sensory systems in the rat become functional at different times. Onset of function follows an invariant sequence believed characteristic of all warm-blooded vertebrates. Among the systems with earliest onset are the vestibular, tactile, and olfactory senses, which develop prenatally. In contrast, onset of function for vision and audition is delayed until about two weeks after birth.

Tables 1 and 2 summarize the timetable of development of the rat's sensory systems. Table 2 also lists some available techniques for functional testing, which are discussed later.

In addition to onset of function, it is necessary to recognize two more principles of sensory development: Onset prior to functional maturation and Achievement of developmental endpoint.

Onset of function precedes functional maturation in all sensory systems. Maturation always involves some degree of improvement in sensitivity, range, or sophistication of sensory processing. Tactile sensitivity, for instance, spreads across body regions, beginning at the snout and working back. Rate of improvement is therefore a better measure of tactile development than is onset of function.

The endpoints of function, such as degree of visual acuity, are the levels of sensory function reached with maturation. Figure 21 depicts maturation curves for two hypothetical sensory systems. The curves are drawn to depict differences in onset and rate that meet at equivalent outcomes.

Table 1

Basic Structure of Sensory Systems

<u>Modality</u>	<u>Receptor</u>	<u>Receptor Location</u>	<u>Input Pathway</u>	<u>Primary area of Central integration</u>	<u>"Higher" Processing</u>
Tactile	Pacinian corpuscles Meissner's cells Ruffini end organs Merkel discs Free nerve ending	Body surface	1) ascending spinal tracts 2) Cranial Nerve V (Trigeminal)	Brainstem: 1) n. gracilis n. cuneatus (trunk region) 2) Trigeminal nuclei (head region)	Thalamic nuclei VPL. VPM Areas 3, 1, 2 of neocortex
Vestibular	Hair Cell	Inner Ear: Semicircular Canals Utricle Saccule	Cranial Nerve VIII (auditory-vestibular)	Pons: Vestibular N. (superior, lateral medial, inferior)	Cerebellum Ascending and Descending tracts
Olfactory	Ciliated bipolar cells	Nasal cavity, olfactory mucosa	Cranial Nerve I (olfactory)	Forebrain: Olfactory bulb	Anterior olfactory nucleus Olfactory tubercle Piriform cortex Amygdala
Auditory	Hair cells	Inner ear: cochlea	Cranial Nerve VIII (auditory-vestibular)	Pons: Dorsal cochlear n. anteroventral cochlear n. posteroventral cochlear n.	Superior olivary complex Inferior colliculus Medial geniculate n. of thalamus Area 42 of neocortex
Vision	Rods and cones	Eye: retina	Cranial Nerve II (Optic)	Lateral geniculate of thalamus Superior colliculus	Area 17 and 18 of neocortex

Table 2

Basic Sensory Development

<u>Modality</u>	<u>Time of Receptor Formation</u>	<u>Time of Central Relay Formation</u>	<u>State of birth</u>	<u>Behavioral testing Technique</u>
Tactile	Merkel Disc: Head E17-17.5 Trunk E 18.5-19	N. cunneatus & N. gracilis: E13-15 Caudal Trigeminal: E13-15 Rostral Trigeminal: E13-17	Functional	Reflexive reactions electrical shock Thermotaxis Placing reaction
Vestibular	E14-20	E12-15	Functional	Righting (aerial, surface) Geotaxis
Olfactory	E11-adulthood	E14-P90	Functional	Orientation tests Preference tests Sniffing
Auditory	E12-14	E12-14	Not functional: first function Day P9-10	Startle reflex Preyer reflex
Visual	E17-P4-5	E17-P6	Not functional: first function Day P6	Phototaxis Optomotor response Visual cliff

E = Embryonic

P = Postnatal

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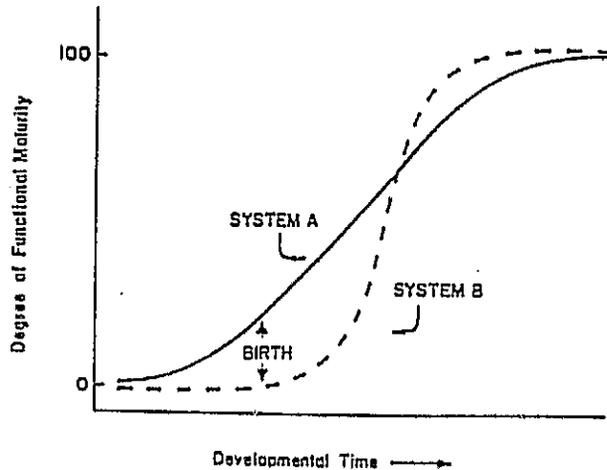


Figure 21. Graphic depiction of functional maturation in two, hypothetical sensory systems. Maturation can differ in onset, rate (slope) and level of final function.

Onset, rate, and final level can vary independently. Since each parameter affects the amount and quality of sensory information collected from the world, each is necessary for a thorough assessment of sensory development and for interpreting the development of complex behavior.

If the predictions of the neurobiologists prove true -- if basic developmental processes remain intact in space -- we must nevertheless remain circumspect for space-related effects on the development of specific sensory mechanisms. For instance, the optical properties of the vertebrate eye are determined, in part, by the properties of their fluid media. Space-related changes in maternal or offspring fluid/electrolyte balances might therefore affect the developing system.

In the rat, and all other vertebrates, the vestibular system is one of the earliest sensory systems to form. It begins to function during the fetal period, well before birth. Thus, pregnancy in space means vestibular development in the absence of gravity.

Several neurobiologists interviewed agreed that vestibular function was another candidate for developmental alteration in space. Development in space may eliminate stimulation to the developing vestibular system -- stimulation that is inherently and invariably present during development on earth. This is a form of stimulation that the buoyancy of the uterine environment will not eliminate. For instance, part of the vertebrate vestibular function involves the stimulation caused by calcium crystals that normally rest on a bed of hair cells in the inner ear. These crystals, the hair cells, and the nerves that project from them to the brain become functional during the fetal period. Null gravity could

eliminate the tonic stimulatory effects of crystals on developing hair cells. Without this stimulation, the system could have a new and unpredictable developmental course. Although genetic mutations have been used to probe into this developmental process, these are imperfect and incomplete approximations to the kinds of effects to be found in space.

BEHAVIORAL DEVELOPMENT

On earth, pups start behaving in a mobile, complex social manner at about 2 weeks. Their locomotor movements have advanced from twitching, to crawling, to walking and climbing. They recognize littermates, learn and remember, develop circadian rhythms, play and begin to develop independence in many spheres of life. By the third week, they are capable of living on their own.

Throughout this early period, the amount and kind of interactions experienced by the pups are important for the formation of social attachments and for the determination of their later social and sexual behavior. To imagine placing this complex of activities into a 0-g environment and predict the consequences is impossible. Our questions must be answered empirically.

Vocalizing. Beginning around Day 5, pups begin to vocalize, or "cry," when they are isolated or cold. These cries are largely composed of frequencies around 40 kHz, which for humans are ultrasonic. For the mother rat, however, the cries are near her peak range of sensitivity, and some researchers believe they attract her to the pups.

Complex behaviors is not a precise category, but it acknowledges a characteristic of the species. Rats form social relationships that influence learned processes, finding food, regulating energy balance, and engaging in behavioral sequences such as play, nest-building, and parental behavior.

PHYSIOLOGICAL DEVELOPMENT

The digestive physiology of the rat pups is initially adapted to a high fat, low carbohydrate milk diet. By the middle of the second week, however, GI physiology starts adapting to solid food, which for rats is high in carbohydrates and low in fats. During this phase, the kidneys begin to concentrate and filter urine and the brain switches irreversibly to aerobic metabolism. These changes and many others are linked to a complex, finely-tuned maturational process and may be disturbed by null gravity.

Thermoregulation. In addition to mechanisms for thermoregulation provided by the huddle, pups can increase heat production chemically by activating special stores of "brown adipose tissue." Around Day 10, shivering becomes a means of thermogenesis, and fur and insulative fat begin to develop. The ability to attain and maintain

normal body temperatures is crucial to survival and growth as well as for more subtle, but vital developmental processes. Both direct and indirect effects of space conditions must be anticipated and measured.

Sexual differentiation, which is both a physiological and a behavioral process, begins during the last week of gestation and continues during the first postnatal weeks. Normally, secretions of the fetal and neonatal gonads govern differentiation, although before birth, maternal secretions can have profound effects.

Sexual maturation. The rate of sexual maturation is significant to both organismic and population biology and should be a standard parameter of space biology studies. For rats, the rate of sexual maturation is regulated to a large extent by olfactory cues, such as urine-borne molecules emitted by sexually active adult males. The molecules can accelerate maturation in female pups by 25% relative to controls. Rats usually mature sexually around the 7th week.

Habitat design can determine the concentration and longevity of the behavioral and olfactory cues that affect sexual maturation. These biological variables must therefore be considered when designing and testing different space facilities. Interpretive confusion can be minimized and effective husbandry maximized by attending to details that can have significant impact on reproduction and development.

NEURAL MATURATION

Space research can help reveal relationships between brain and behavior. Zero gravity provides an opportunity to study a "natural" alteration in both structure and function while preserving the overall integrity of the organismic system.

The neurobiologists I interviewed -- Almlil, Keefe, Oppenheim, Souza, Teitelbaum -- agreed that massive changes in neural development were unlikely in zero-gravity. There is no reason to suspect that gravitational forces affect most of the basic processes, such as cellular birth, migration, differentiation, and synaptogenesis. Nevertheless, some of these scientists believed that discrete aspects of neural development might be susceptible and that space offers the perfect tool for investigation. On Space Lab-3, for instance, Souza will study the embryonic neural tube, the precursor of the brain (see Appendix J).

Dr. Ronald Oppenheim (Bowman-Gray Medical School) believes that the development of neuro-muscular junctions and muscle spindles might be affected by the absence of gravity. His reasoning is that these systems may use the gravitational forces or their stimulating effects on muscle tissue as part of the natural, formative process. According to this speculative but fascinating logic, development in space would remove this organizational force from the developmental

process. Such an "experiment" has never been conducted on earth. It cannot be. Evolution, during its many millions of natural experiments and millions of generations of replications, has always had available the stimulating presence of gravity for the formation of muscle-nerve systems that control movement (i.e., compensate for gravity). It is possible that Mother Nature, with her characteristically opportunistic style, has evolved the use of gravity itself as part of this ontogenetic process. This is a difficult and elusive idea -- one which has not frequently been entertained. One reason is that, on earth, we cannot escape gravitational influences and such questions cannot be asked empirically. But a space station will change this and we can begin to enter such new realms of inquiry.

Studies of neural development relevant to space problems can resemble methodologically much of modern neuroscience (see Appendix K). Unfortunately, commerce regulations have prohibited the transport of some isotopes and fixative to the U.S.S.R., where they are not readily available. Consequently, some of the U.S. experiments on Cosmos biosatellites have been compromised. The existence of a U.S. space program could raise immediately the technical level. Many neural parameters are available for measurement: transmitter distribution levels, enzymes, and structural characteristics of the brain. Standard laboratory techniques must be analyzed carefully for application in space. A space station will permit many studies that cannot be performed on short or unmanned flights.

MATERNAL BEHAVIOR....ON EARTH AND IN SPACE

An important component of the mammalian reproductive cycle is the set of caregiving activities that ensues after birth. In Norway rats nearly all of these activities are performed by the mother, we will deal with maternal behavior rather than with the broader category of parental behavior.

Maternal behavior is more than motherlove. For the newborn and developing rat pups, maternal behavior comprises most of their environment, providing heat, tactile stimulation, nutrition, and protection. At every level of organization, genetic information, energy, and environmental factors interact to shape and guide development. The process of development cannot be understood without knowledge of the environment. During the early life of most rats and most mammals, parental behavior is the most important environmental factor.

To study the effects of null gravity and other environmental conditions of space on development, we must separate two influences: (a) effects of space that act directly on the developing organism and (b) effects of space that act indirectly by altering maternal

behavior. Indirect, or secondary, influences are likely to be as great or greater than direct, or primary, influences. For reasons of both pure research and for practical aspects of animal management, we must tackle these issues quickly and forcefully.

MATERNAL BEHAVIOR OF THE RAT

The relationship between maternal behavior and offspring development represents a classic dyad. The litter can be considered one "unit" of the dyad and the mother the other "unit." Together they comprise a complex developmental system, which we can characterize and analyze using standard analytic approaches to dyadic interactions.

Measures of rat maternal behavior. Maternal behavior consists of several categories that are normally performed in relation to the young and their needs. Decades of research has led to the adoption of a fairly standard profile of rat maternal behavior, consisting of four activities: (1) nursing, (2) nesting, (3) licking, and (4) transporting. Figure 22 depicts these four maternal activities.

These maternal behaviors are organized into a cycle, which normally lasts 3-4 weeks. Throughout the cycle, the quantity and intensity of each of these activities changes in an orderly manner. Figure 23 shows the cycle in graphic form. A circadian rhythm is obscured by the daily averaging used to produce the smooth curves shown in the figure. The diminution of each activity follows an orderly timecourse. These changes, however, are regulated, in part, by mutual (dyadic) stimulation between mother and offspring.

1. Nursing and Lactation are salient parts of mother-litter interactions. The nursing dam usually lies on her side with the pups lined up along her ventrum (Figure 22), or she "crouches" above them. The pups use olfactory, tactile, and possibly vestibular cues to orient toward, search for, and attach to the nipples. They suckle vigorously, even when milk is unavailable. Suckling stimulation has two effects on the mother's hormonal systems.

The long-term effect is exerted primarily through regulation of the hormone prolactin in the mother. Suckling increases and maintains high prolactin levels, which increases the mother's metabolic rate and her food intake so that she produces enough milk to feed the growing litter. When the pups begin to nurse less and thus stimulate the mother less, prolactin levels decline, as does milk production.

The short-term hormonal effect of suckling stimulates the release of another hormone, oxytocin, from the dam's pituitary gland. Oxytocin is carried to mammary tissue, where it stimulates contraction of myoepithelial tissue, causing the "letdown" of milk to the nipples and then into the suckling pups. In response to the

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pulse of milk, the pups respond in unison with a dramatic "stretch response."

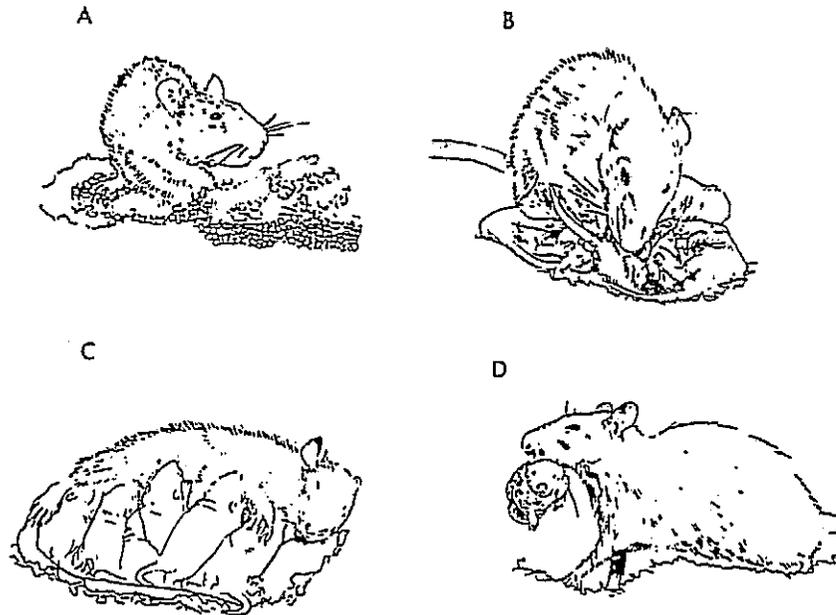


Figure 22. Maternal activities in the rat. (A) Nest-building, (B) Licking, (C) Nursing, (D) Retrieval (transport).

For at least the first two weeks of postnatal life, pups derive all of their nutrients, water, electrolytes and antibodies from the mother's milk. Weaning, the transition from reliance on mother's milk to independent ingestion of food and water, is gradual and can last for an additional two weeks or more. By the time pups wean the dam is usually feeding a biomass of offspring that nearly equals her own body weight (see Figure 26, below). It is therefore understandable that dams often triple their pre-pregnancy food consumption.

2. Nesting. This category includes the non-nutritive contact interactions between mothers and pups that occur in the nest. Prior to Day 12, all mother-litter contact is initiated and terminated by the dam. Contact is established when she approaches the nest and joins the huddle and it lasts until she terminates the nesting bout. The frequency and duration of these visits are greatest early in the lactational cycle, and decline after about the first week. In addition, nesting contact is greater during the light portion of the day than at night, when the dam spends more time feeding.

Nesting is an excellent measure of maternal behavior. Numerous devices can be used to record the quantity and pattern of maternal nesting. For the Cosmos '83 experiments, we have designed and built a dual chamber Rat Maternal Behavior Cage (Figure 24). The cage is

divided into two sections. The smaller side is used by the mother as the nesting area because of its size and overhead protection. She uses the larger side for rest, relaxation, feeding and drinking. The cage design requires that the mother cross the wall to join her litter. Her body weight depresses the floor under the nest and activates a microswitch, read by a computer. The floor is counterbalanced to accommodate changes in the weight of the mother and the litter. A thermister under the nest records nest temperature. Above the pups, a microphone detects their high frequency calls. These signals are recorded and averaged every 15 minutes. To enter their own feeding cage, the pups must leave the nest. It is therefore possible to measure separately ingestion by the mother and by the litter. These cages are used in conjunction with time-lapse video tape recordings. Video records show that mother rats spend 95% of their time in the nest in direct contact with the young. The video record also reveals the number and pattern of milk ejections. During nursing bouts, these milk ejections occur about six minutes apart.

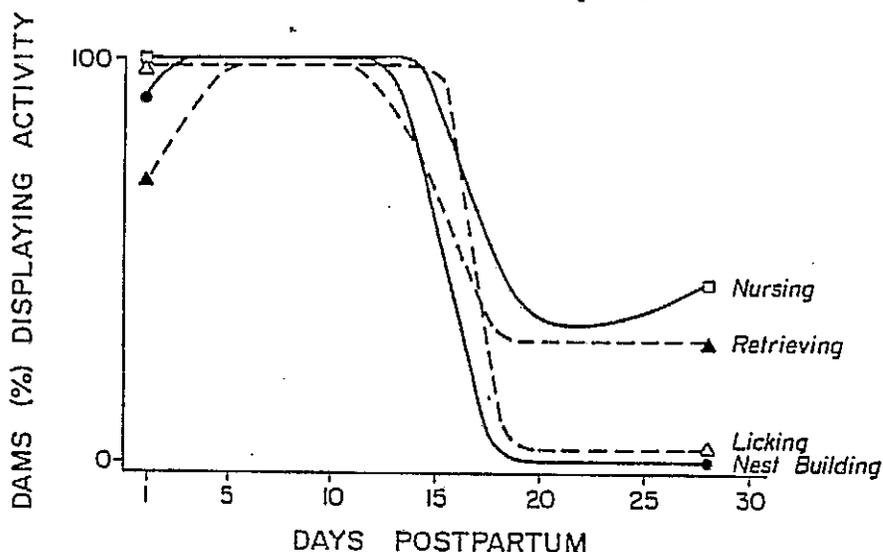


Figure 23.
Maternal behavior cycle

3. Licking. Licking is another important component of maternal behavior and can also be seen and scored from the video record. There are several forms of licking. Pups receive tactile and temperature stimulation (cooling) when licked by the dam. Much of the mother's licking is focused on the infant's anogenital region. The contact activates the "micturition reflex," which voids the pups. Recent research has also shown that the mother ingests their urine. At early ages, pup urine is dilute (hypotonic) and is a useful fluid source for the mother. Licking the pups and ingesting the urine allows the rat dam to reclaim about two-thirds of the water she has transferred to her pups via milk on the previous day.

Male pups are licked about twice as much as females because of a testosterone-dependent substance in their urine. There is evidence that anogenital stimulation has a masculinizing effect on the recipient. There are long-lasting behavioral consequences of this

differential treatment. Males receiving less than normal amounts of anogenital licking show less robust sexual behavior than brothers that were licked more. It appears, then, that the full expression of sexual differentiation in rats is dependent not just on the way hormones act on brain and body tissue, but also on how they change the mother's behavior, and thus change the world in which the infant develops.

The immediate and long-term roles of licking are a good example of how carefully documented behavioral observations can be used to assess developmental effects in complex interactive situations. The important mechanisms are sometimes subtle and the consequences of early events often reverberate through subsequent developmental stages.

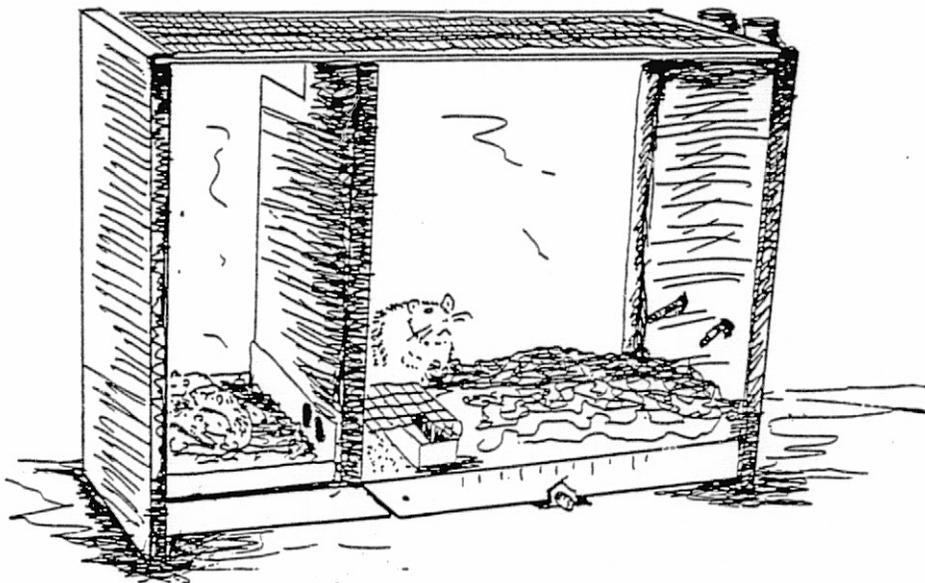
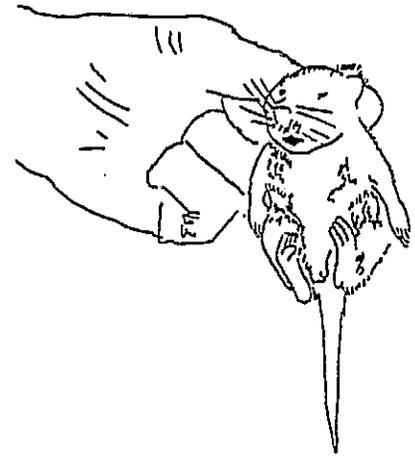


Figure 24. A specially-designed, "dual-chamber" cage used to measure components of the dyadic interactions of the rat mother and her offspring. female's immobility

4. Transport behavior is a common form of parental behavior in many animals. Rat dams lift their pups by the skin of their dorsal neck or back and transport the pup in their mouth. As pups become larger and more mobile, mothers stop transporting them. To some extent, pups reduce the energy expended by the dam during transport. Beginning at about one-week, pups adduct their hindlegs, extend forelimbs to their body, and tuck their tail (Figure 25). When lifted, this "transport reflex" is stimulated by tactile cues to the back and by the vestibular or proprioceptive cues from lifting. The developmental appearance of this reflex matches the

Figure 25. The "transport response" of the rat pup. The pup tucks its limbs in response to tactile stimulation to the neck and back, as well as being lifted. It thereby facilitates the mother's transport.



dam's propensity to display transport. Then, as her maternal behavior wanes, so does the pup's reflex. Its expression is timed to its usefulness (see Figure 36, below).

These simple transport responses present a fascinating model of development and of ontogenetic coordination that could be studied in a space station laboratory. How "adaptable" is the mother's behavior? Will she continue to transport the pups without negative feedback of increased weight? How will the pups' reflexes develop in the absence of the gravitational constraints on transport? Will transport be abandoned entirely in favor of a functionally similar solution? Alternatively, will transport function maladaptively because the mother or her pups do not adapt to this unique environment?

5. Hyperphagia and energy balance during lactation. Figure 26 shows the growth curve (total body weights) of a litter of eight rat pups. All energy that enters the litter from conception to about 15 days postpartum is mediated by the mother's metabolism. After birth, the energy demands of their growth and thermoregulation are enormous. To meet these demands and to maintain her energy homeostasis, the lactating dam must go into metabolic "high gear." The dam's food consumption can triple relative to her pre-pregnancy rate. By Day 15 postpartum, the weight of the litter as a biomass can equal that of the dam. This underscores the enormous energetic load of lactation.

Basic metabolic measurements of mammals during flight have not yet been made (see interview with Dr. Schatte). We must construct complete energy budgets for adult males, females, juveniles, as well as pregnant and lactating females. These conversion values (energy ingested -- into organismic tissue) are important for the numerous biological studies of function described in this report and could

allow us to compare the costs of raising and maintaining different species in space.

Studies of body composition after null gravity, centrifugation, and forced immobility have provided a unique perspective on the nature of physiological regulation on earth and, in so doing, have made important contributions to the foundations of space biology. Unfortunately, the database is still meager and, in particular, we need fuller metabolic measures, long-term exposures, in-flight studies, and behavioral correlates. Clearly, a space station is the crucial ingredient.

The next studies of body composition must investigate female body composition in relation to space, ground-based model systems, and hypergravity. Similar investigations should be conducted on the pregnant and lactating animal since their profile and responsivity is often different. A space station will permit new and needed investigations of major physiological adaptations to long-term exposure, which cannot be studied on the short U.S. flights.

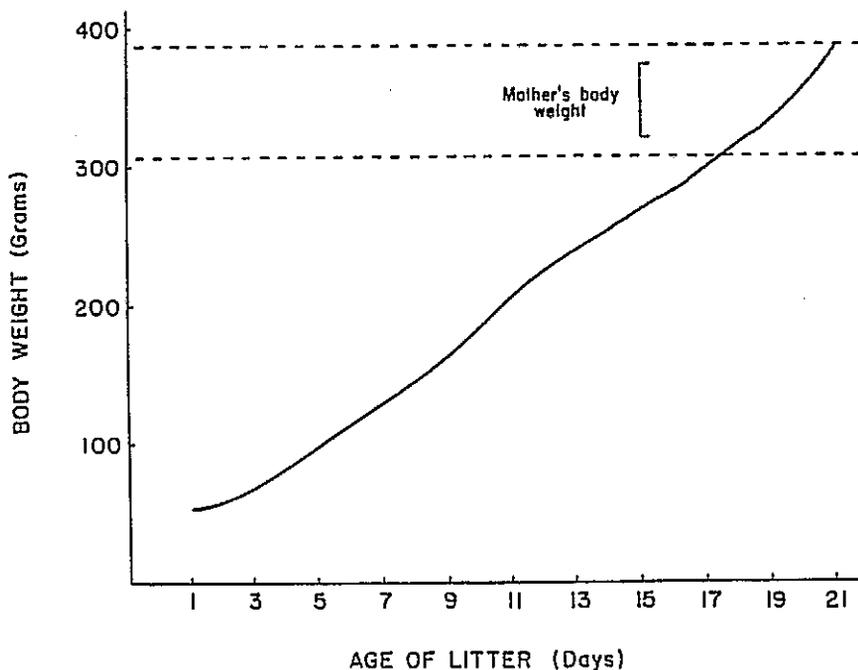


Figure 26. Growth curve of Norway rat pups (whole litter of eight). The dashed line shows the approximate weight of a single mother. All of the food energy for growth is provided by mother's milk.

DYADIC INTERACTIONS REGULATE MOTHER-LITTER RELATIONS

Mother-litter relations in the rat represent a classic dyadic interaction. The litter, a behavioral and physiological aggregate, regulates and is regulated by the behavior and physiology of the mother.

Maternal licking serves the pups' metabolic and hygienic needs, arouses them behaviorally, and induces them to nurse and thus obtain more water, electrolytes, and nutritive energy. The mother receives tactile stimulation from the pups that coordinates her milk letdowns, regulates her prolactin levels, and adjusts her feeding and lactational metabolism.

During mother-litter contact, body temperature is regulated. Net flow of thermal energy changes direction during the first three weeks of postnatal life. Initially, thermal energy flows primarily from the mother to the litter. After Day 10, the flow becomes more dynamic and bi-directional until, eventually, the direction is reversed and nest visits are terminated when heat transfer from litter to the mother raises the dam's temperature.

Breakdowns or perturbations in the dyadic system must be carefully studied. Recognizing that the mother fails to attend to the pups is not enough. Since maternal behavior is partly controlled by stimuli such as the infants' odors and behavior, we must determine whether the dam is receiving sufficient stimulation. Similarly, if the pups are not developing normally, the problem may lie with the parental behavior. Both sides of the dyad must be monitored.

Without appropriate analytic tools, however, these problems defy understanding. One of the simplest and best methods is "cross-fostering," a procedure which exchanges parents and offspring. Figure 27 shows a simple cross-fostering arrangement between Parent A and Offspring A with their counterparts Parent B and Offspring B.

The beauty of the cross-fostering paradigm lies in its application to situation involving an "unknown" unit. In Figure 27, Parent A and Offspring A have just returned from space. Even in the comfortable confines of the terrestrial lab, the dyad is doing poorly. Parent A neglects the Offspring, which are immobile and non-sollicitous. Are both Parent and Offspring debilitated from the space exposure? Possibly. However, we can investigate with the simple cross-fostering manipulation shown in the figure. We know that Parent and Offspring B are healthy. We therefore pair the "known" healthy parent with the questionable Offspring A, and the known Offspring B with the unknown Parent A, and measure. The dyad is analyzable!

Note that the cross-fostering procedure requires "known" dyadic

units. Until we "know" a great deal about behavior, physiology, and reproduction in space, we will be forced to rely upon ground-based or centrifugated "knowns" for these analyses. This is yet another reason for us to hurry and acquire basic knowledge of mammalian function and adaptation to space.

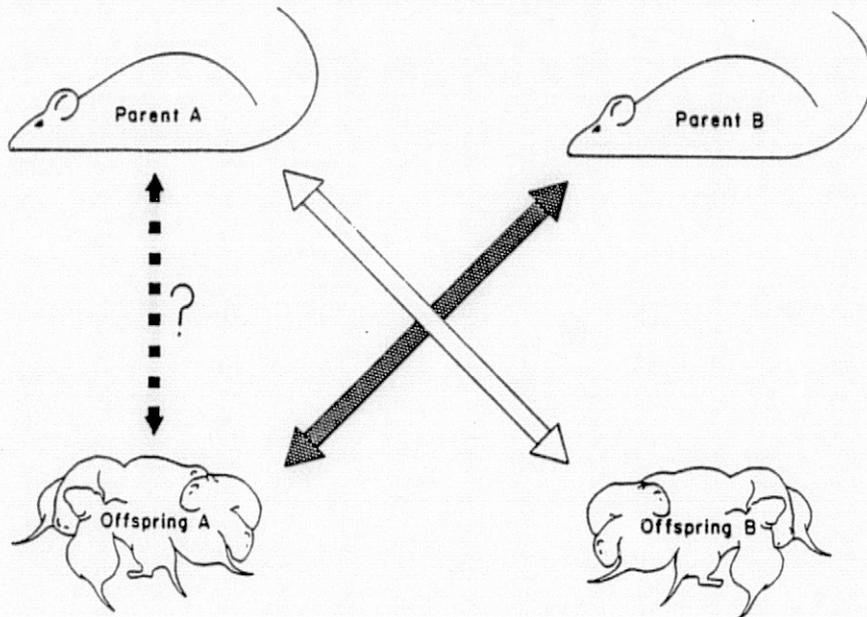


Figure 27. The Cross-fostering Paradigm.
A valuable tool for dissecting dyadic
effects.

A PROGRAM FOR STUDYING DEVELOPMENT AND MATERNAL BEHAVIOR IN SPACE

Every expert who I interviewed about rat development and maternal behavior agreed that null gravity will eliminate the physical coherence needed for normal expression and coordination of mother-litter interactions. They envisioned problems at every stage of development: brooding, nursing, licking, the formation of attachments and weaning. Figure 28 depicts the chaos they envision.

It is possible, of course, that we have a "geocentric" view of the limits and process of adaptation and the role of gravity in it. Rats may adapt readily to space conditions.

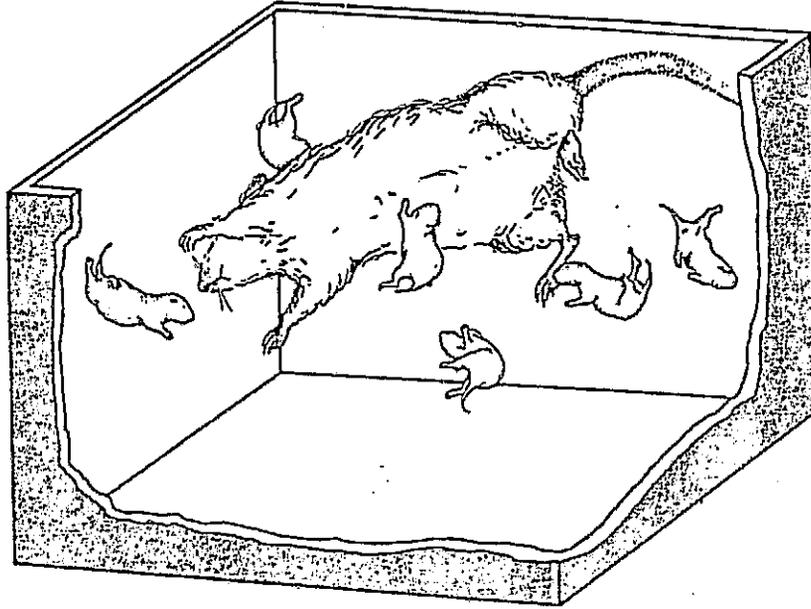


Figure 36. A mother rat and infants at null gravity.

Most experts agreed that gravity and vestibular cues are crucial for early interactions. Successful birth and postnatal development in space will require special approaches to avoid situations such as the one in this drawing.

Nevertheless, NASA needs to make a good first impression on its constituency and on itself when it studies, finally, mammalian development in space. If we don't want the public to see dead infant rats floating in a chamber, NASA's first study should not be too bold or blindly goal-oriented. Our first pioneering ventures can, and should be, important, stimulating, exciting, and successful.

Although the U.S. can make good use of the Cosmos and Space Lab flights to collect short-term data, full studies of early postnatal development and maternal behavior should await a space station with experts on board who can insure the safety and well-being of the animals. Meanwhile, we can continue to refine initial research questions, giving particular attention to critical ground-based research and developing space-related techniques and equipment.

As a preliminary study, we can fly pregnant rats, improving upon Cosmos '83 research. Given current limits on our knowledge of mammalian reproduction and development in space, flying pregnant rats and having them give birth on Earth will probably be more successful than trying to solve problems of both birth and rearing.

The following improvements should be made in the design of the Cosmos post-flight studies:

(a) visual records should be collected;

(b) the shape and surface area of the flight compartment should be redesigned to offer a more graspable surface area and a smaller surface/volume ratio (a ratio of at least .60 should be achieved rather than the .06 of the Cosmos compartment);

(c) some rats should have pre-flight experiences which include familiarization with compartment and other rats as well as habituation to diet and mode of feeding;

(d) the animals should have free access to food and water during flight; and

(e) a cross-fostering design should be used so that flighted females rear ground-gestated pups and flight-gestated pups are reared by ground-control females.

The aims of these initial studies are:

1. To collect basic observational data on behavioral and physiological responses to space conditions, particularly responses of females mammals.

2. To determine whether or not mammalian gestation can proceed in space.

3. To evaluate flight compartments in terms of safety, support and stress management for the rats.

The overall success of animal research in our space program may depend upon efforts to understand the needs and preferences of pregnant and lactating animals for niches in space. On Earth, these habitats are similar to those inhabited during non-reproductive phases. Assessments must be made in space. If our studies of niches in space show similar results, we will gain a more basic understanding of female rats' needs and preferences.

Systematic analyses of space habitats for rats will be evaluated by measuring adaptation and stress, studying the outcome of pregnancy, and evaluating behavior during and after flight. Controls will include ground-based groups, on-board centrifuged, and non-pregnant equivalents.

In addition to evaluating designs that maximize graspable surface/volume features (as in the sample habitats shown in Figures 13 - 17), I recommend using the "rat as design engineer" principle discussed earlier. In the present context, pregnant rats could be flown in individual compartments, some of which are equipped with

material into which the animal can dig a burrow and maternal den. The late-pregnant animal normally displays increased levels of nest-building. Figure 29 shows an experimental burrow and a "control" compartment with an empty module in place of the burrow area.

Habitat engineers should be encouraged to consult with biologists -- and with the rats -- to incorporate biologically-derived configurations. For instance, before rats will breed in burrows on earth, they must have at least two "bolt holes." In space, rats may have strong preferences for different features. They may require one or three holes, narrower alleys in the burrow, or more sidearms. The self-creation feature of the habitat depicted in Figure 29a provides an efficient means of gaining insight into the rats' needs and preferences that could make the difference between breeding success and failure. In order to evaluate whether specific findings or conclusions regarding habitat effects are real or imagined, an identical environment could be used as a "control" condition (Figure 29b). This self-creation approach could also be used to evaluate other features of the habitats.

The specific measures will depend upon the goal of the study: hormone levels, circadian rhythms, feeding, or reproductive performance could all be measured to indicate adaptation rate. Pregnancy outcome will be an important issue.

The next goal of analysis may be to extend exposure to space conditions during pregnancy. To reduce the risk of resorption and unsuccessful implantation, first attempts should involve mid- to late-pregnancy. Exposure can then be extended to include more stages of pregnancy. We will discuss such plans, below.

Nesting at null gravity. In terrestrial habitats, gravity, in combination with the infants' tactile and thermal responses, keeps the huddle coherent and organized. The huddle is a behavioral and physiological entity serving thermal, metabolic, and social needs. Moreover, the mother's behavior and mother-litter interactions depend on the ability of the mother to interact with the litter, particularly on her ability to nurse and brood. In turn, patterns of maternal care are coordinated by stimuli delivered by the litter as a unit. Thermal balance, for example, regulates the mother's nest visitations, and total suckling stimulation regulates her hormone levels.

Nursing at null gravity. Since the pups may be floating randomly, there are more ways to imagine breakdowns of the dyadic relationship than there are ways to imagine it functioning smoothly. Lactational physiology might fail if null gravity effects body fluids, electrolyte balance, calcium metabolism, or hormones and muscles. As discussed earlier, the pups may not be able to nurse if null gravity disrupts the approach, contact, and orientation responses upon which their nursing behavior seems to be based.

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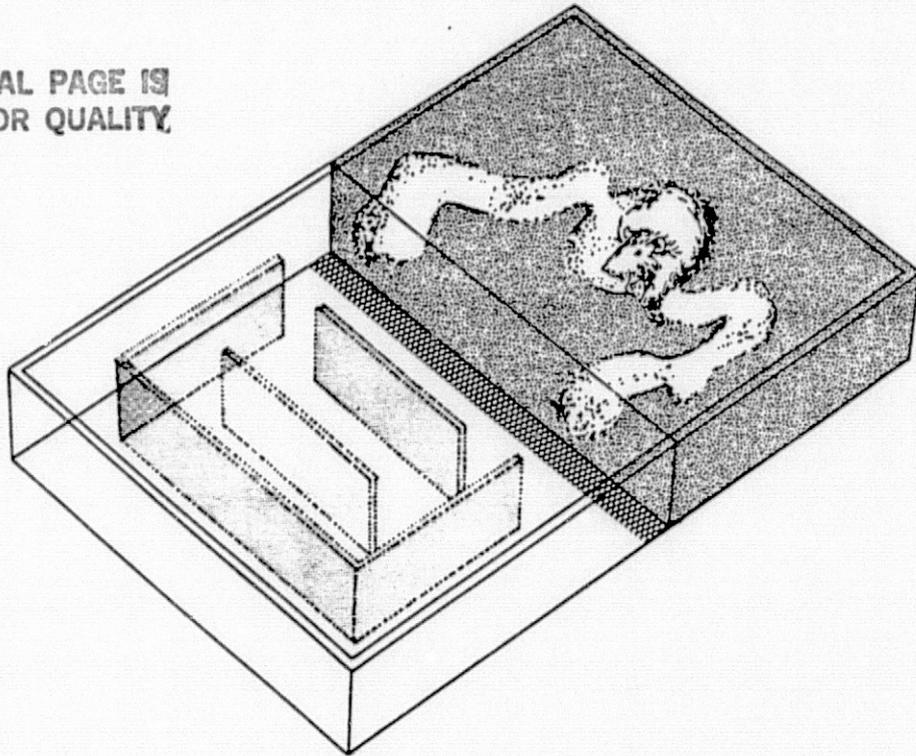
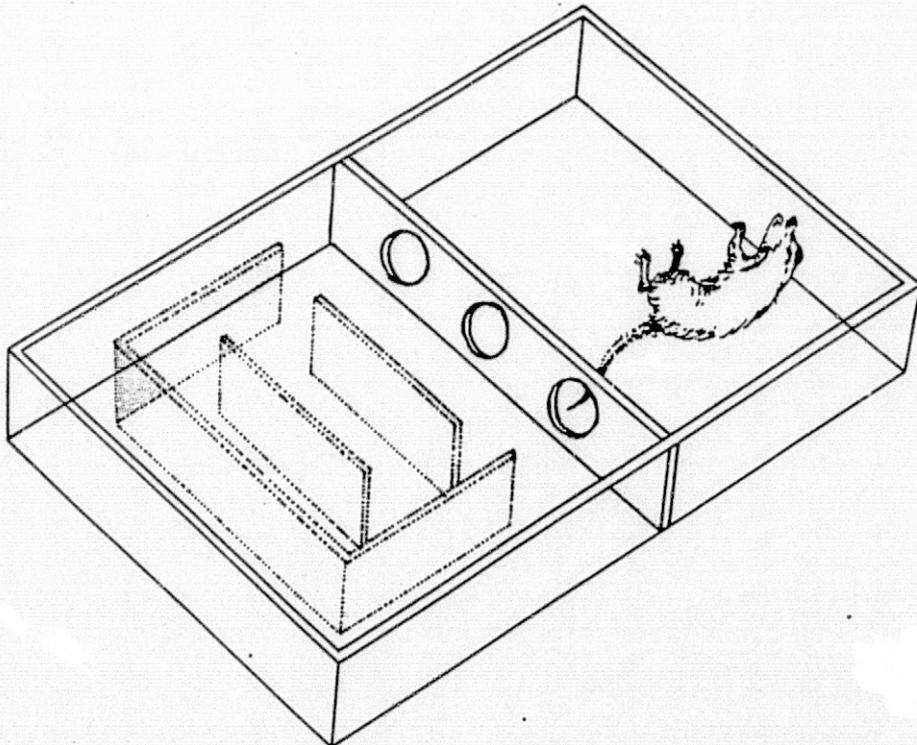


Figure 28. A flight habitat for a pregnant rat. Similar habitats, differing in crucial features, can be compared in terms of the adaptation of the inhabitants. This way we can determine the important environmental variables and assess the value of data from preference tests.



DESIGN OF A NATAL NEST FOR SPACE

This plan is presented to illustrate possible solutions to a problem which is, at present, hypothetical. I reiterate that NASA should not attempt to have rats deliver and rear their offspring in space before gaining additional knowledge and learning to manage healthy rats in space habitats. This brief example is offered as a parallel and supplement to the more detailed discussions in the Reproduction section, which are largely applicable here.

Precursors. The first attempts to have rats deliver and rear litters in space should be preceded by:

- (1) acquiring a database on understanding mammalian physiology, particularly female physiology, at null gravity,
- (2) a thorough picture of behavioral and physiological adaptation to null gravity, including comparisons of level and rates of adaptation as a function of habitat characteristics,
- (3) successfully supporting complex social and physiological interactions in a space environment, such as mating and pregnancy in space followed by delivery of a normal litter on Earth.

With these criteria satisfied, we can move on to the next phase -- rearing space babies from conception to birth and then through the developmental and reproductive cycles.

The goals of our research program depend upon our achieving reproduction in space, but they ultimately concern the application of this ability to the study of problems of life processes such as development, organismic function, adaptation, and evolutionary change.

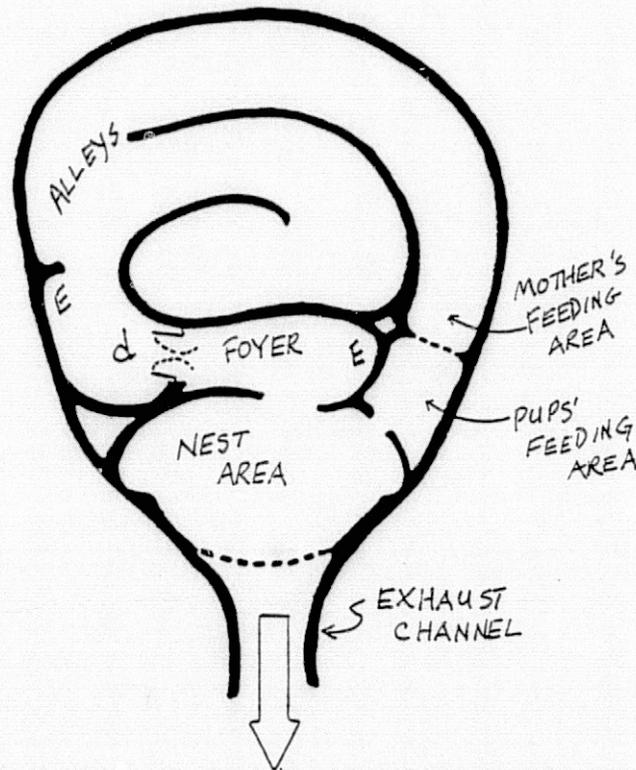
Many of the principles of empirical approach discussed in the Reproduction section are directly applicable here. A similar program of ground-based research should be established in anticipation of the needs of the space station program. One useful tool here might be borrowed from the approach delineated earlier -- namely, the use of vestibularly disrupted animals. Their maternal behavior is notoriously poor (a fair warning?). It has recently been found in France, however, that smaller, bowl-shaped nests help keep the mother-litter dyad together and survival is reinstated in vestibular mutant mice that normally fail to rear their litters.

Figure 30 shows a cross section of a natal nest that I envision for delivery and early postnatal care of the young in space. The habitat is designed with several unique, problem-solving features.

The nest area is partially bounded by a perforated surface that provides guided airflow into the Exhaust Channel, which operates

under regulated, negative pressure. The purpose of this arrangement is to create a zone that can contain the pups as an aggregate, as shown in Figure 31a. This will allow the pups to orient toward and contact each other and provide the mother with a localized, litter-aggregate to approach and attend. The configuration of the nest area is designed to encourage, but not force, containment. I envision the air currents to be adjustable (by the specialist working in the space station) and maintained at a level that maintains the aggregate, but permits them to move away from it and to explore. A modest thermal gradient increasing toward the nest would provide additional assistance for orientation.

Figure 37. Cross-section of a natal nest for rats in a space station.



The FOYER to the nest is specially-designed to function as a "locked" area from which the mother, but not the pups, can exit and return to the nest. This is accomplished by the spring-loaded swinging doors, depicted at "d." In order to open these doors, it is necessary to brace against Surface E and exert the necessary force. Only the mother's body is sufficient to span the distance, and it is a natural response for her to make. Bracing spots are provided for entry and for exit. Once the mother leaves the nest area, she has available a burrow-like habitat formed of alleys and

den areas. The feeding area for the mother is arranged near a screen. This area is adjacent and open to the pups' feeding area. The pups can enter their feeding area through the small opening shown in the figure which is designed to exclude the mother. Two adjacent feeding areas separate maternal consumption from that of the pups (for metabolic and developmental tests), yet preserve the social stimulation that is normally available that aids the pups normal weaning onto solid food (depicted in Figure 31b).

This habitat, as shown here, is designed to embody the principles that I have elaborated upon in earlier sections of this report. It features small Surface/Volume ratios, separation of measures for analysis of dyadic relations, concerns for coherence and encouragement of group relations, and maximal visibility for study and continued refinement.

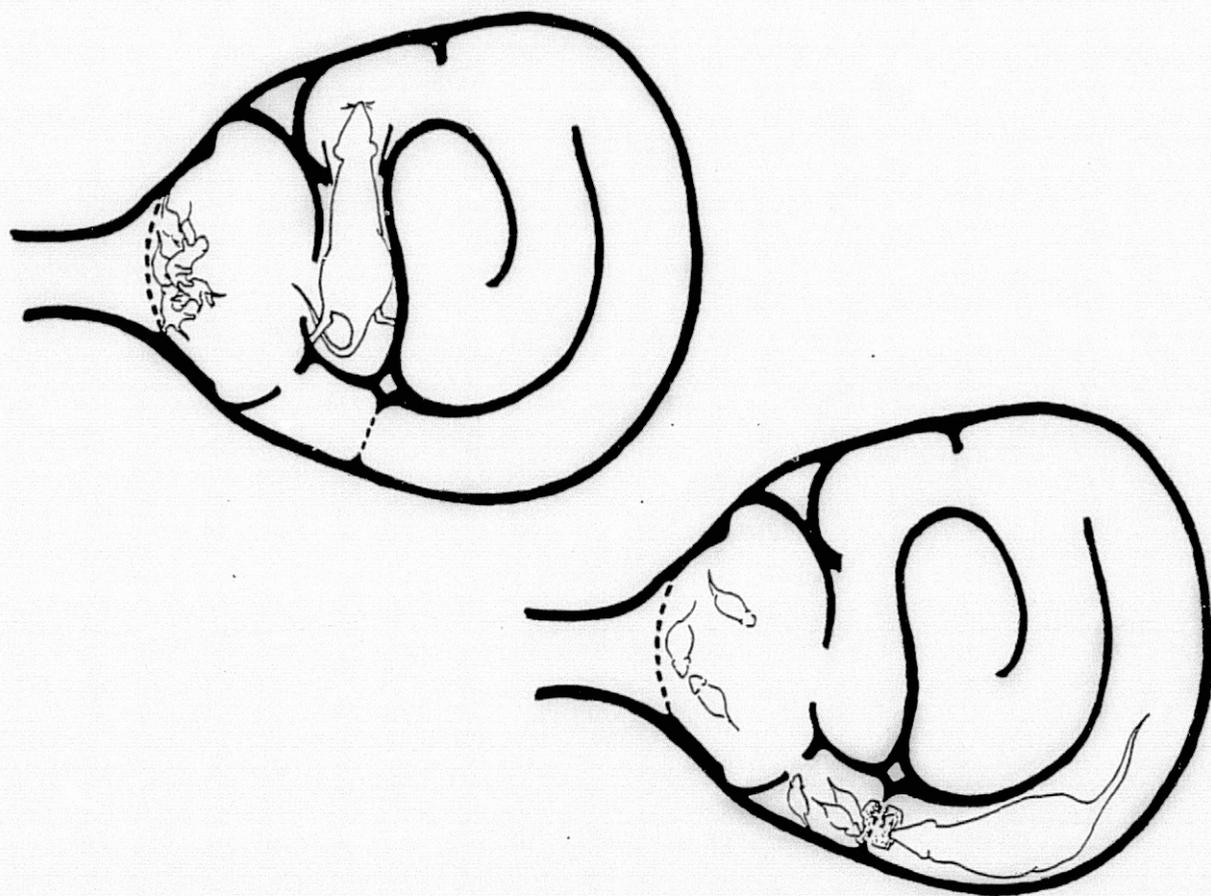


Figure 38. The natal nest in space, (a) showing a mother leaving the nest area where her young litter is residing. The aggregate is maintained by gentle air currents that exhaust through the channel behind the nest. Moderate temperature gradients, cooling away from the nest may also be used to encourage the pups to orient in and around the nest. The dam is using the spring-loaded doors, which she can move because she can brace her body against a wall for leverage. (b) The natal nest also allows pups and mother to feed separately for metabolic studies, while providing pups with the social cues from the mother that guide them to feed.

POSTNATAL STUDIES IN SPACE

The accomplishment of adaptation, mating, fertilization, gestation, birth and rearing make available sequential generations of animals for a new realm of space biology. The sequence of problems and accomplishments required to establish mammalian reproduction in space will itself identify scientific questions which are not part of our current perspective. Undoubtedly, our sophistication and views will develop with experiences required to accomplish initial tasks.

But even from our current position, we can anticipate numerous questions for which to prepare. If space affects the mother and/or the offspring, we must determine whether duration of exposure or period of exposure is involved. The experimental designs shown in Figures 32 and 33, below, can be used to evaluate their relative effects.

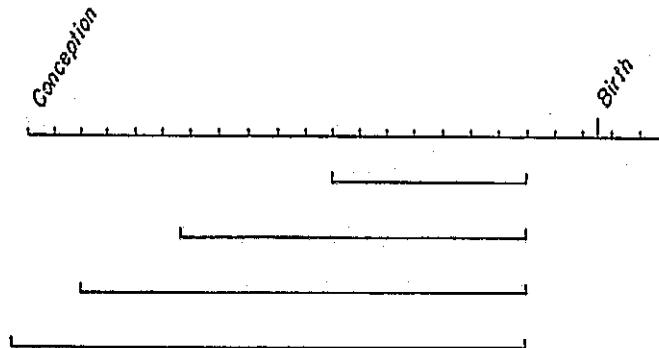


Figure 39. Graphic depiction of how duration of exposure can be gradually expanded to encompass an increasingly broad range of developmental stages.

Foremost among the inevitable questions will be the normalcy of offspring conceived, gestated, and born at null gravity. By the time this full cycle is achieved, there should be few managerial surprises since each stage will have been previously accomplished, but independently. The unique opportunity here will be to test the idea that, in combination, each stage still functions adequately. As each stage is added to the sequence, we face exposures to space conditions of increasingly longer duration. Thus, it is necessary to separate developmental studies of "critical periods" from those of cumulative, longitudinal effects (see Figure 33, below).

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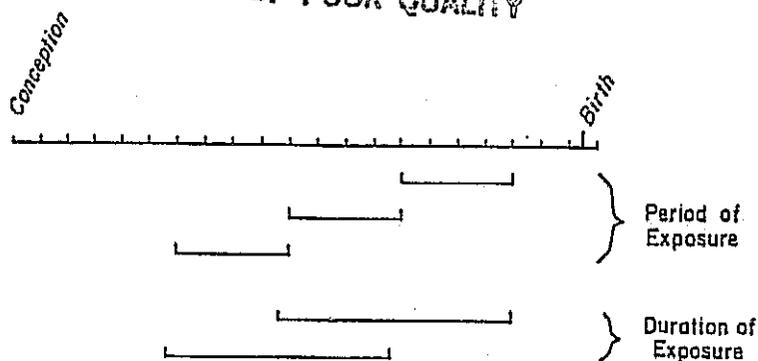


Figure 40. Graphic depiction of different experimental paradigms used to examine exposures to space of identical lengths that occur at different developmental stages, vs those of different lengths that encompass similar stages. By such comparisons it is possible to separate effects that are due to duration versus those to a "sensitive period".

Alternative species. The use of species other than the Norway rat as flight subjects will provide an important parallel approach. The Norway rat, the common house mouse (Mus musculus), as well as gerbils (Meriones maniculatus) and hamsters (Mesocricetus auratus) have short gestation periods of 16 to 22 days and give birth to immature young. In contrast, a small number of species have long gestation periods of 42 to 65 days and give birth to exceedingly well-developed young. These "precocious" species include the common guinea pig (Cavia porcellus) and the more exotic Egyptian spiny mouse (Acomys cahirinus). Since Acomys is smaller than the guinea pig by a factor of 20 and is more closely related to rats and mice, it permits rigorous cross-species comparisons and is easier to manage.

The Egyptian spiny mouse (Figure 34) gives birth to only two or three young after a 42-day gestation period. The newborn has a full coat of fur, functional eyes and ears, and walks within hours of birth. Although the neonatal spiny mouse nurses, it is not an obligatory suckler, as are its immature cousins.

Precocious species, such as the spiny mouse, are important candidates for studies of the effects of space on later stages of in utero development than are possible with rats and common mice. Eventually, we can use comparative methods to examine the effects on equivalent stages of development. For instance, maturation of the visual apparatus at null gravity can be compared when the eye develops in the uterus versus outside the mother.

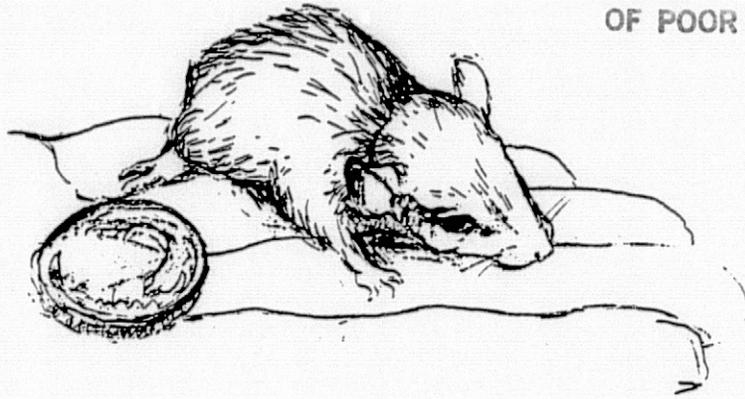


Figure 29. A newborn Egyptian Spiny Mouse, drawn from a photograph of a pup just six hours after birth. This precocious newborn has open eyes and ears. It is fully mobile soon after birth. It is in a human hand, with a penny as a reference to show its minute size.

Measures of Development

The postnatal development of mammals conceived, gestated, and born in space is the focus of research discussed in this report. These efforts deserve NASA's commitment because of the important contributions they will make to basic and applied science, to the cultural pride of our nation, and to the perpetual stretching of human imagination and destiny.

Evaluation of these first space babies will require a thoughtfully planned program. I have recommended that we begin this important and vital task by flying pregnant mothers and returning the unborn subjects to Earth for study. This, of course, is the design of the Cosmos '83 experiment, mentioned above. By way of illustration, I will review briefly the specific tests that are available to evaluate neonates. Many of these are part of the neuro-ontogeny experiments of Cosmos '83.

Initial postnatal examination. In order to minimize disruption to newborns and mothers, the tests will require little time. The critical variables are: a) body weight, b) body length -- crown to rump, c) number of living pups, d) gender composition of each litter, and e) ano-genital (AG) distance.

Ano-genital (AG) distance warrants brief explanation. The distance, in fractions of a millimeter, from the anterior border of the male's rectum to the base of his phallus predicts the degree of

sexual differentiation. Prenatal factors, such as maternal stress, can feminize male offspring. Drs. Ingeborg and Byron Ward of Villanova University (Appendix F) have shown that AG distance is a good quantitative measure of sexual differentiation.

Morphological markers. During the course of daily weighing and testing, basic morphological markers, supplemental to the variables mentioned above, will be recorded: f) development of fur, g) separation of external ear (pinna) from head, i) unsealing of ears, j) opening of eyes, k) eruption of teeth, and l) vaginal opening/testicular descent.

Motor and reflex development. A variety of simple and non-invasive tests of the rat pup's motor and reflex competence can be used to evaluate physical development and help interpret the results of other tests, such as those requiring a motor response.

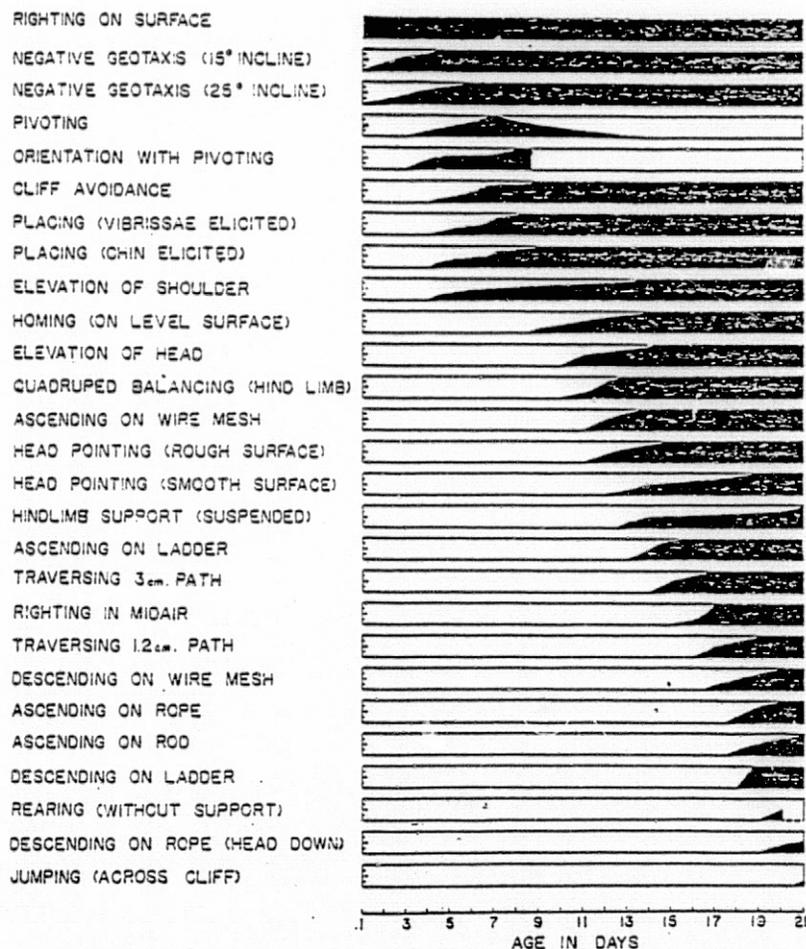


Fig. 22. Summary diagram of the emergence of different postural, locomotor and related skills. In the majority of instances performance level (0, 25, 50, 75 and 100 per cent) refers to the percentage of animals successful in the full display of the response. In a few instances the reference is to level of performance with respect to asymptotic response frequency.

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Observation yields a great deal of information. Each day during early life, pups are videotaped on a flat surface in a warm environment. Figure 35 depicts the definite and orderly sequences of postural and movement development. The film and video tapes can be shared with other laboratories that analyze the data with different methods.

Simply lifting the rat pup can be used to test reflexes. By grasping the pup at the nape of the neck, we expect to see the "transport reflex." Figure 36 shows the normal ontogenetic appearance and diminution of the reflex. An organism whose development is disrupted by space flight might fail to develop this adaptive reaction or may not lose it at the appropriate stage.

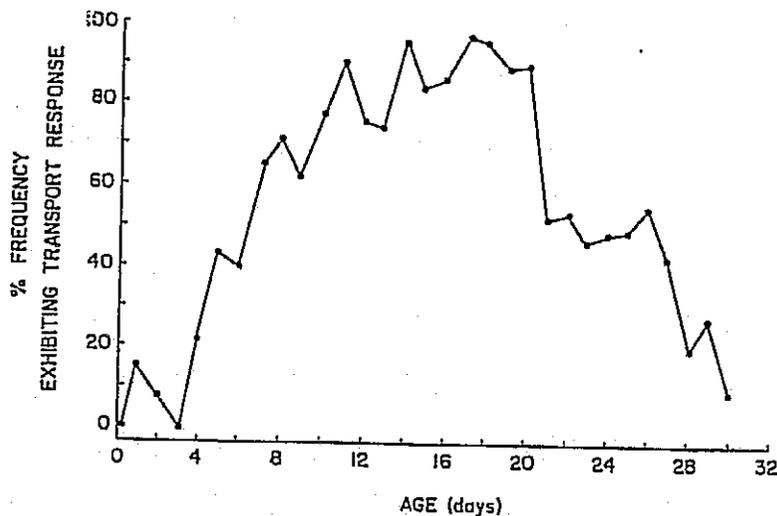


Figure 31. Developmental appearance and disappearance of the "transport response" of rat pups.

Sensory Tests

The tests described below are not an exhaustive set but have been selected to determine (within fairly narrow but practical boundaries) whether sensory function is intact. The interpretation of such data will be discussed in the section immediately following the survey.

Tactile sensitivity is tested with a calibrated, flexible strand (von Frey hair). The resting pup is stimulated, usually around the snout and rump. Behavioral orientation -- turning, withdrawing, head-raising -- is used to map sensitivity across the body.

Vestibular sensitivity. Most developmental neurobiologists that I interviewed bet that space will effect the vestibular system during gestation. A number of test can be used to assess vestibular function, including:

a) surface righting - a reflexive response elicited by placing a pup on its back. From birth, the infant demonstrates its ability to detect vestibular perturbation by struggling to "right" itself. Its efficiency improves rapidly with motor development.

b) air righting - a better test of vestibular function than surface righting. Animals in an inverted position reorient in mid-air when released. Acceleration cues (vestibular stimuli) replace the tactile stimulation received when the animal rests on its back for the surface righting test (Figure 37).

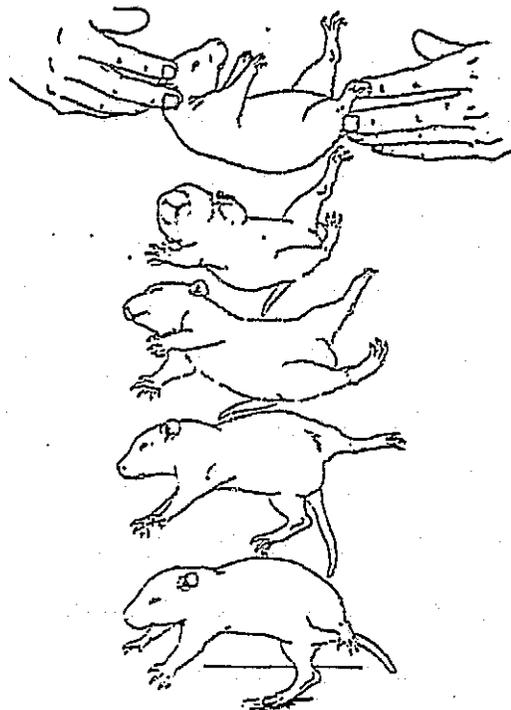
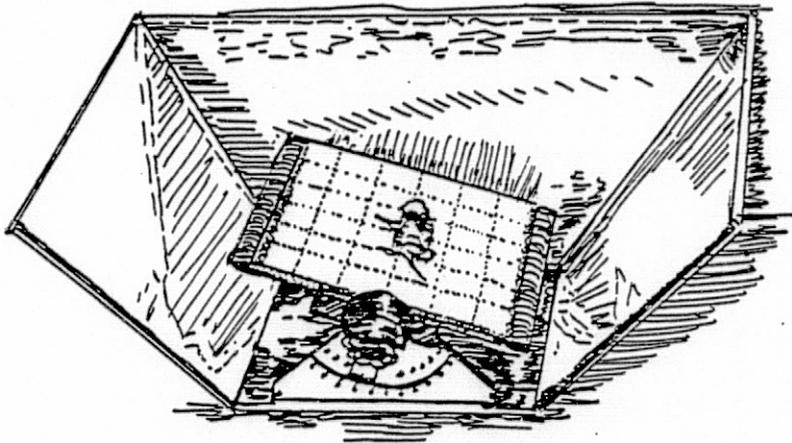


Fig. 37. Tracings of righting in mid-air; speed 64 frames per s. Rat aged 17 days.

c) tilt table - Many infants, including rats, show a reflexive response, called "negative geotaxis," or a tendency to move against an incline (away from gravity). For rats, this unlearned reaction may be masked by other reactions when they develop spontaneous mobility around Days 7-9. Before then, however, a tilt table, such as the one shown in Figure 38, can be used to ask whether the young vestibular system can detect

various degrees of incline. To interpret the results, motor responses must be measured to determine whether the subject is sufficiently strong and motorically competent to orient. The apparatus shown in Figure 38 was specially designed to give three simultaneous views of a tilted rat -- from the top, the front, and the side -- and thus increase our ability to recognize reactions that indicate vestibular detection of tilt.

Figure 33. A tilt-table used for testing the ability of infant rodents to detect angular tilt and display a "geotaxic" response.



d) Rotating stage - Rat pups can detect acceleration, as evidenced by an unconditioned tendency to turn their heads against the direction of rotation. Since the test is new, nothing is known about the parameters of the pup's response other than that it is no longer reliable by Day 7 and that it disappears as the juveniles gain more mobility.

e) swimming test - orientation in warm water is eliminated with vestibular dysfunction. Postural adjustments are different in vestibularly-disturbed animals when support cues are removed by the buoyancy (see Schallert interview, Appendix G).

Olfaction. Recordings of respiratory rates, transduced by a strain gauge plethysmograph, provide the most precise and quantitative measure of olfactory function in rat pups since detectable odors elicit a burst of increased respiration, or polypnea (Figure 39).

This technique is useful for several reasons. First, it is

based on a natural, unlearned response; teaching newborn rats takes time and the animal can outgrow the experiment before testing. Second, the technique is not physically demanding, can be administered quickly, and is performed in a warm chamber, factors that all minimize stress. Finally, respiratory measurements taken before an olfactory stimulus is presented, provide a useful baseline index of "tonus," of breathing strength and neuromuscular lability, and of the animal's level of arousal. Each of these baseline measures can be compared to age-related norms.

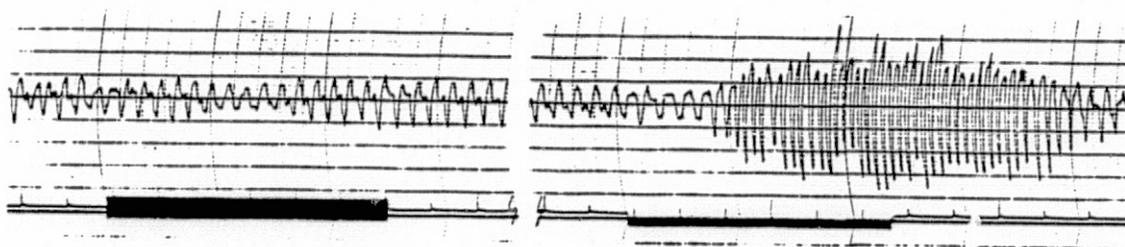


FIG. 2. Respiratory record of a 10-day-old presented with a clean air control injection (double deflection) that did not significantly alter respiration and, later, a 6 sec pulse of an odorant injection that induced polypnea.

Audition. The simplest measurement is detection of a tone: The simplest index is an unconditioned twitch, called a startle reaction or Preyer reflex. Like the olfactory test, and others, this measure of audition has the advantage of being administered quickly and is based on unconditioned responses. The pup's detection response can be measured on a ballistic stage or any movement-sensing device.

It is not sufficient to use the morphological event of ear-opening to mark onset of auditory function. The pups' ears unseal at about 12 days of age, but there is measurable auditory function prior to their opening. Ear-opening and auditory system maturation can be independently accelerated or retarded.

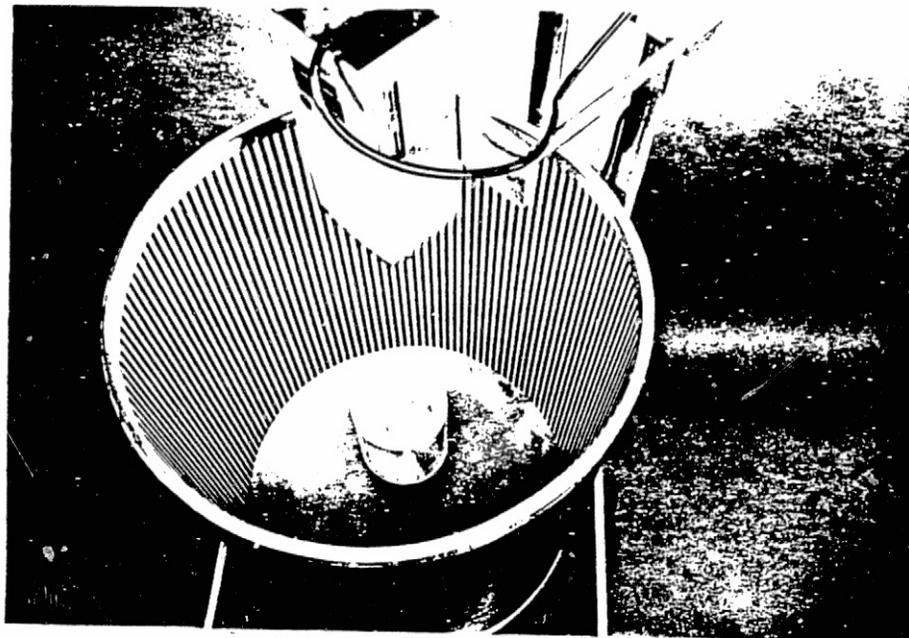
In general, the auditory system first becomes sensitive to low frequency tones and then gradually to higher frequencies.

The biggest developmental changes occur between Days 12 and 20 and therefore using two or three different frequencies at a specified amplitude (db) provides a representative picture of auditory sensitivity. Since rat audition is broadly tuned and extends into ranges that are ultrasonic to humans, acoustic signals ranging up to 40 kHz (human sensitivity drops off after 20,000 cycles) can be tested.

Vision. Although vision is not as important to rats as it is to humans and other primates, it is used to detect movements of potential predators as well as for navigating. Several methods can be used to test visual acuity in rats. One of the best methods tests the unconditioned "optokinetic reaction," a reflexive turning of the head or body in response to a moving visual array. Figure 39 show the interior of an "optokinetic drum," within which a visual array is slowly rotated. An animal is place in a clear central chamber, and its reactions are observed or filmed for analysis. The limits of visual acuity can be estimated by reducing the size of the stripes until the

Figure 35. Interior view of an optokinetic drum.

A single pup is placed in the small central chamber from where it views the rotating, striped walls. If the visual system can resolve the stripes, the pup displays an unlearned, optokinetic response. By increasing the spatial frequency of the stripes until the response disappears, visual acuity can be measured. acuity can thus be measured.



response disappears. This test does not provide information on other important aspects of visual perception, such as depth perception, accommodation, or visual discrimination, but it is a simple and accurate tool for investigating the ability of the eye and of peripheral aspects of the visual system to resolve edges and estimate limits in spatial frequency.

Development of Complex Behaviors

The newborn rat, despite its extreme immaturity at birth, is more than a passive recipient of maternal care. From the beginning of postnatal life, its sensory functions and motor abilities are integrated into sequences of complex behaviors that are much more than discrete, reflexive reactions. These complex behaviors are directed, maintained, and used by the infant to adapt to its postnatal world.

Nursing. Immediately after birth, nursing is probably the most salient complex behavior. The means by which infant rats attach to the nipples is well understood. Dimethyldisulfide (DMDS), found in the saliva of lactating rats and in rat amniotic fluid, is deposited on the mother's nipples during self-licking. DMDS is one of the important odorants that activates the pups and stimulates their rudimentary movements toward the dam. Tactile cues, mediated by various branches of the facial and trigeminal nerves, add fine-tuned direction to the pups movements. Their final orientation involves vestibular cues. The pups often turn onto their backs under the dam's ventrum and orient upward. Although olfactory cues are necessary for early nursing, they are not sufficient. Vestibular cues also appear important throughout the sequence of behavior used to orient toward the mother, move along her body, locate and grasp the nipple, and settle into a nursing posture. Proprioceptive cues, emanating from the pup's body, may also be important for coordinating movement and orientation. Tactile stimuli, particularly in the snout region, are necessary for "rooting" and grasping the nipple. Not surprisingly, these sensory systems all develop early, at least on Earth.

Systematic tests of the sensory, motor, and motivational bases of suckling use an anesthetized, lactating dam. Without the normal interactions and assistance of the mother, the behavioral competence of the pup can be seen in its purest state. These test can be transferred to postflight tests to determine how sensory, motor, and integrative processes are coordinated into complex, adaptive behavior in a neonate.

At about the same time as eyes open and physical prowess and stamina develop, a variety of complex behaviors emerge. Because their maturational processes are related, isolating these behaviors into discrete behavioral categories and describing their developmental emergence is difficult. Nevertheless, we

can note some of the behaviors as well as when they first occur in a typical laboratory on Earth.

Sexual and parental behavior. The emergence of sexual and parental behavior can be regarded as the final phase of a developmental cycle that began with the same events in the parental generation six weeks earlier. We have already noted developmental precursors and determinants of sexual behavior, such as licking, maternal stress, nutrition, and social experience. Parental behavior has not been studied as carefully, but I have tried to show that it is amenable to standardized and quantifiable tests and can therefore be evaluated after exposure to space.

Feeding and drinking. Independence is based in part on the pups' need for more nutritive energy than their mother can supply, as well as on maturational processes. Their first bites of solid food usually occur around Day 15 in social settings. Drinking typically follows feeding.

A number of standardized tests measure various aspects of the weaning process. Some are observational. Others are manipulative and involve choice tests that allow the pup to demonstrate a preference for suckling or feeding. Other tests measure dietary preferences and the ability to regulate the homeostatic balance of specific nutrients, fluids, or energy levels.

Huddling, or social contact. When social groups are not feeding, mating, fighting, or playing, they spend most of their time in huddles, engaging in social contact behavior. This form of affiliative behavior indicates social preferences and attachments. A variety of standardized tests can be used to assess social preferences and the strength of such attachments. Since attachments are established through identifiable developmental events, they can be used to understand the kind and amount of complex social learning that has transpired developmentally.

Social play. Generally, social play emerges around Day 15 and reaches feverish levels around Day 25. Most interactions recognized as social play involve episodes of "play fighting" -- explosive bursts of running, jumping, spinning, leaping, and pouncing. Social play, which occurs with no discernible trigger, is regulated and patterned. According to some researchers, males engage in more rough play than females.

Tests of social play assess the formation and maintenance of dominance relations, of sex-typical social patterns, of social motivation, and of contingent behavioral reactions. Most of these tests can be quantified and conducted under standardized test conditions. In order to observe robust levels of behavior, these tests are usually designed to minimize stress.

Learning and memory. . Experimental psychology has long been adroit at testing the ability of rats to learn and remember. Recent methodological advances have added to the list a number of impressive tests of neonatal learning abilities. We can therefore evaluate the impact of rearing conditions in space on the ability of a developing animal to learn, remember, and integrate complex information into new patterns of behavior.

The big leap will come when we tackle the problems of managing and investigating postnatal development in space.

THE FUTURE

How will "normalcy" be defined and measured in our space-reared rats? One way, of course, is in relation to terrestrial life. "Normal" would be defined as similar to norms for terrestrial life. But is this too geocentric? Perhaps we should reset our standards and work within a framework of viability and capacity to reproduce in space. We should prepare ourselves to discover that a "normal" space baby develops at different rates, and in different sequences than its terrestrial counterparts, but that it shows the same signs of biological adaptation as we find on Earth. Such ideas could shake fundamental concepts of evolutionary biology. Genetic expression may be linked to environmental forces such as gravity in ways that we have not imagined. Molecular transcription could alter in space, or the fundamentals of epigenesis could vary -- resulting in new genotype-phenotype consequences. Clearly, it is premature to dive into these issues, but it does not take long to feel the edges of our terrestrial imagination.

The first generation studies will be devoted to observation, description, and evaluation. The particular challenges will be determined by what we find. A space station should be equipped, at this point, to handle the kind of dynamism that typifies pioneering science at its best. Workers should have the resources to change plans and designs, work in new ways, and respond to new discoveries and insights. The value of hands-on presence of scientists is enormous when treading in these unknown areas. The excitement of science comes largely from the human factors.

Once we are able to rear animals that are adapted to this "novel" habitat, we may have to develop new models and/or theories of organismic adaptation. There will be data from cellular, systematic, and organismic levels of analysis. We will be able to test some of these ideas by playing the process "backwards." We can study the process of adaptation to gravity in organisms conceived and reared in space. Again, the combination of possible results are staggering. Indeed, such

possibilities could be analyzed by bringing together specialists from different fields for a Study Section to explore the potential of two such symmetrical populations -- organisms brought to space from earth, and their counterparts that are transferred to earth from space. As these developmental studies become more accessible, the value and role of genetically homogeneous organisms will become more important. Behavior genetics should thus be viewed as a relevant discipline to this scientific trajectory.

On the horizon are multi-generation studies. At the point at which we can bring animals into space and allow them to fill the new niche with sequential generations of offspring, we can truly begin to study the big biological questions of adaptation and the organization of mammalian life processes. Until that time, we will be working with organisms that bring to space a direct legacy of gravity. Our first adult rats will have lived on Earth with our gravitational forces. Their offspring will be initiated from zygotes that were formed and organized by cellular processes that operated in the presence of gravity and within a larger physiological system governed by gravity. In the multi-generation studies, we may find new worlds of biological organization waiting to be unleashed and to unleash our views of our origins and our future.

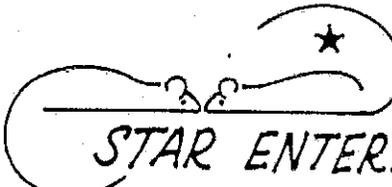
PART IV

APPENDIX

EDITED INTERVIEWS:

- A. Natural History of the Norway Rat, B. G. Galef, Jr.
- B. Group Mating in Rats, M. McClintock,
- C. Techniques for Movement Analysis and Vestibular Testing of Neonatal Rats. C. R. Almlı
- D. Rat Experiments of Cosmos-1129, J. R. Keefe
- E. Stress and Pituitary-Adrenal Function, S. Levine
- F. Perinatal Stress and Its Effects on Offspring and Mother, I. & O. B. Ward
- G. Vestibular Function and Measurements in Rats, T. Schallert
- H. Development and Reproduction in Space, C. Schatte
- I. Studies of Primate Pregnancies in Space, E. Sackett
- J. Neuroembryological Studies in Space, K. Souza
- K. Neuroembryological Effects of Space, R. Oppenheim

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Research Director

NATURAL HISTORY OF THE NORWAY RAT (RATTUS NORVEGICUS)

An interview with B. G. Galef, Jr., Ph.D. (McMaster University)

Conducted by J. Alberts

- A. What do we know about the origins of the Norway rat?
- G. The basic story is that during Paleolithic times, members of the genus Rattus were fairly common throughout Europe. They appear to have become very rare because there is no mention of them at any point in the Bible or in Greek and Roman literature. There is no word in Greek or Latin for rat, while there is some mention of smaller rodents, such as mice. The genus Rattus first appeared in Western Europe, the first recorded year in literature . . .
- A. 600 A.D.
- G. And Rattus rattus, the black rat, spread very rapidly throughout the bubonic plague period, the 14th century. They were subsequently displaced by the Norway rat throughout Europe. Norway rats arrived in the States in 1775, that is the earliest mention of them.
- A. Was Rattus norvegicus the carrier of bubonic plague?
- G. No, Rattus rattus was probably the carrier of bubonic plague. Anyway, they (Rattus norvegicus) were here in 1775, spread west with the westward expansion of the pioneers, arrived in California with the 49ers, and gradually filled up the whole continent. Anywhere where you have permafrost, they don't make out very well. Nevertheless, they are found in Alberta. There aren't very good figures that I know of for the U.S., but in similar climatic zones in Germany, about 60% of the rats are human commensals and 40% live in the field.
- A. So, the fascile statement in one of Romer's books, namely that Rattus norvegicus originated in China, is not necessarily true.

G. My guess is that nobody knows. Rats seem to be of European origin. But no one can be precise. I don't think that this is different than with other common species. To locate where they originated is difficult. Some sort of Indo-European origin is, I think, appropriate. If you want to know if they are closely associated with people, they surely are. Their spread throughout the world is very closely correlated with human movement. The arrival of both Rattus rattus and Rattus norvegicus on various South Pacific islands, Australia and elsewhere is highly correlated with the arrival of industries.

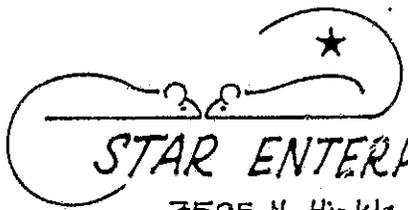
A. And chances are that their appearances around Europe was also linked to human travels?

G. Yes, I read somewhere that the Rattus rattus moved in with the Mongolian hordes drifting through Northern Europe from the Asian portion of Europe and the Soviet Union. But all this is lost in antiquity; we are stuck with the problem of either finding mention of them in literature, or not finding mention. For instance, because there is the story about the piper of Hameln, we know that there were rats in Hameln in the 16th or 17th century. Because Dr. Johnson mentioned a place where you can buy rat poison, you know that they existed in his locale. Unfortunately, even in modern times, there's a lot of confusion about terminology. Terms such as "Mus rattus", make it very hard to know whether or not it is rats or mice.

The situation is further clouded by negative attitudes that can lead to reluctance to acknowledge them. For instance, rats are not mentioned in the Bible . . . what does that mean?

A. What about the history of domesticated rats or albino rats?

G. Again, it is somewhat lost in history. Curt Richter claims that domesticated rats originated from rats kept outside sporting establishments where rats were thrown into a pit with a pit terrier. People would bet on how long it would take the dog to kill the rats. These were betting establishments. I don't know the exact date, but the general picture was that this was popular for about 100 years and then it was outlawed. They collected the rats for the sport, and funny looking rats, white ones, black ones and so on, were maintained so that when people saw them, they would remember the place. There is also on record somewhere, around 1840-something, an Italian who was reputed to have domesticated rats.



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GROUP MATING IN RATS

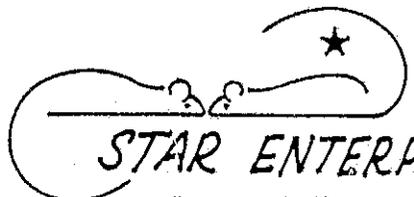
An interview with Dr. Martha McClintock (University of Chicago)

Conducted by J. Alberts

- M. If a female is in post partum estrus, and if in her environment there is another female that is both pregnant and lactating, she won't conceive and implantation will be delayed and she'll be generally miserable. Now the way the group mating works is they get pregnant, they mate in groups, but then they don't carry in groups, they are separated.
- A. Do you think that if they were together in groups, such an arrangement would inhibit the pregnancies going to term?
- M. It's possible. We haven't worked out the timeframe, but if they are staggered at eight day intervals, then there is suppression. But if they're all time-mated, so they're all. . .since you are starting at day 12, which is post implantation, my feeling is that you might be OK.
- A. That's my hope, too. But I thought that because they are a group during the flight, that it would be better for them to be familiar with one another, rather than suddenly placed in a novel group.
- M. Absolutely. If they're going to be together, yes, definitely.
- A. One of my suggestions to the Soviets was that they should have a group of 15 females housed together; hopefully they would start cycling together. Then introduce into that group, 5 or 6 males and let them mate. Twelve days later select the ten animals that are the most obviously pregnant.
- M. If you really care, I would go in and take complete control and do it time-mating.
- A. And mate them individually?
- M. Yes. If your goal is to get ten animals that are within a day of each other, I would start with a colony of 50, smear them, and then on the basis of the smears, mate them with males.

- A. And then pair them? Or this colony of 50 is comprised of females that are living in group cages?
- M. No, I did mean not living together; they could all be singly-housed. That would make smearing easy.
- A. You have them singly-housed, you smear them, they mate, then start forming the cohorts at that point.
- M. Then start forming the cohort. It's a tradeoff, because you know, there they are just newly pregnant, meeting new females and they may abort.
- A. Yes, that's why I was going the other way on it.
- M. Well, I guess then what I would do is, I guess I'd be nervous that you don't have a large enough colony. Just as you said, you were saying groups of 15, do they have to go up with a group of ten?
- A. Yes.
- M. Living together?
- A. Yes. In a cage that's about 22 by 8 inches by 6 1/2 inches.
- M. Are you going to have a control on earth?
- A. Yes, two control groups on earth.
- M. I guess there are certain limitations. So that's a given. Then I think that the way that you said is the way to go, and I would just go on. If you have all 50 together in a group they're not going to get to know each other anyway.
- A. No, but even if the flighted cohort consisted of 10 females and five of them happened to have been group housed together before, I think it would be better.
- M. Yes. So what I would do is house them in groups of 8, say five groups of 8 females living together, let them live together for awhile to increase the likelihood of synchrony (but don't count on it, because it runs around 60%). Follow the smears and then, if you wanted to, you could introduce a male and have them mate as a group; that would be possible. Or, you could take them out and just mate them in a cage. The reason for taking them out and mating them in a cage is then you don't have to worry about the subordinates not getting enough. Upon introduction of a male there are going to be all kinds of bizarre dynamics going on, too. That would be the reason for taking them out and putting them with a male and then put them back together. And then coalesce them.
- A. Well, that would be done at least.
- M. I would say coalesce them on day ten.

- A. I was going to say at least two days before flight, and that would be day ten.
- M. I would pick day ten, because it would be two days after implantation but would give you two days before flight.
- A. What about diet, when would you put them on the flight diet if that was distinct from their lab diet?
- M. All along.
- A. Yes, that was my view, too, the sooner the better.
- M. Yes, and the control group should be treated identically.
- A. I agree. Well, I certainly hope we get a chance to do this in the States, because these Soviet experiments are a mess. They have a different attitude.



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Research Director

TECHNIQUES FOR MOVEMENT ANALYSIS AND VESTIBULAR TESTING OF NEONATAL RATS

An interview with Dr. C. Robert Almlie
(Washington University Medical School)

Conducted by J. Alberts

RA. I guess that the most likely effects on the pregnant mother would be electrolyte and body water changes. I would also suspect some profound change in metabolism, basically due to the organism's activity level in a weightless environment. I understand that it supposedly takes effort to perform skilled movements at null gravity. I would anticipate that the organism would adapt to that and try to conserve energy; perhaps there would be more passive floating when it wasn't actively trying to do something.

JA. Do you think it would be profitable for NASA to start systematic, daily measurements of organisms that go up into null gravity and combine films of what they are doing with direct metabolic measurements? The purpose would be to measure adaptation per unit of activity.

RA. Yes, I outlined a few things, and that was essentially the first one that came to my mind: How is an organism's behavior really changed by being in a novel and weightless environment. I think it would be very interesting to know characteristics, exploratory activity levels, metabolism, these sort of things. To me, that would be the first thing I would want to know.

I would think those sort of things would be very interesting to look at, and then, of course, when you're looking at those types of patterns, basic motor types of patterns, it would be helpful to have a couple of rats together to look at their interactions.

JA. Let's back up a bit, so I can be sure I'm thinking of the same things that you are. When you talk about metabolism, are you talking about oxygen consumption and respiratory quotients more than . . .

- RA. The statement I made, you know, about it takes more effort on land to accomplish specific tasks up there, is that basically a truth, do you know?
- JA. I don't know. I'm not sure how precise our knowledge is about this yet.
- RA. It seems to me, I guess, when I was watching them, they would do something that I would perceive of as relatively simple and they had to get this rest period afterwards, you know, when they were doing some of the external types of things. Not when they've done space walks, but when they've come along side of a satellite. I find that I'm at a loss for the terminology. But it was my impression that these sorts of things, dangling out there and trying to get to a specific area and do some types of manipulations and that sort of thing, was physically stressing.
- JA. Regardless of what the human response is, we have to remember that these are individuals that are in space by choice and design, and they're trained. If we're going to fly rats, we may want to know what their baseline adaptation rate is. And I would think also that there are probably some investigations one might want to do about accelerating adaptation, if there is an adaptation period.
- RA. To free associate, you could set up a "conditioning paradise", the sort of thing that requires the rats to go through certain types of changing procedures to get them to go through a process of theoretically elevated energy expenditure. But, again, it's my impression that when they aren't doing something like that, they might actually go to an extreme low in terms of metabolic output, which again could be wrong, but my intuition is that if you're kind of floating around, that's very different than standing or sitting or manipulating your position and that sort of thing. I would think it would be more relaxing.
- JA. It might be, of course they may just be stressed to hell then. I've been wondering if there are any preflight conditions that you could put a rat into that would attenuate this. My prediction is that it would be a severe stress on first exposure. I've been wondering if rats should swim as training, for instance, or be vibrated and swim and just give them a variety of different experiences, some of which might involve changes in proprioceptive feedback.
- RA. Again, my intuition leads me to predict that there would be tremendous differences in motor sequencing and that sort of thing. Motor control and bodily orientation used to accomplish tasks would be very different from the kind you find in gravity.
- JA. I think you're right. This is one area where I think that our areas of specialization have a lot to offer in terms of being attuned to movement, to that level of behavior, i.e., orientation and activity. And, if you put the animals together in a group, somehow things would come out, I'm sure. What are your thoughts about a movement notation system or any kind of a quantitative system.
- RA. Well, I'm biased right now towards the movement analysis we're doing and trying to develop with the premies (premature infants) right now.
- JA. Tell me about it.

RA. Well, what we're basically using is a kind of a modified Eshkol-Wachman notation system. When I was initially learning it, I thought, what a pile of crap. But three years later, I have a different view. One of its benefits is that it's the only movement analysis system I know of that one can look at the "score", it is done graphically, and go through and actually duplicate the movements, exactly the way they were accomplished the first time. Most of the other movement notations cannot come near an exact reproduction, but this movement notation system can. So it seems to me, because it has that degree of replicability, that it would be a good system to try to quantify. Phil (Tertelbaum) doesn't use it quantitatively at all, he basically uses it to focus his attention on repetition and patterns and that sort of thing. He just takes the score and does his own subjective interpretation of the score. And what I'm trying to work on now is essentially a computer analysis system that will turn all of these positions and movements, time factors and quantify them.

JA. What you are doing - is this part of your computerization scheme?

RA. Well, we're right now in Phase I of the program. We are now doing the initial studies --- basically scoring the movements. There is an image processor here that we use for drawing neurons, and I'm now also using it to analyze the movements. Basically, you go through with your high speed film, and with a video camera you take a picture of each one of your film frames and digitize it. Then you play games with the computer and get quantitative characteristics of what moves from one frame to the next frame. No matter how complex the movement is, by going through and subtracting out everything that doesn't move, you get what is left is the image of the total movement over time. We're on ground floor one in this and we've just gone through and found out that it's worth pursuing. So now we're finishing up the scoring.

JA. I think I know your answer to this because you're already investing time and effort into this one, but, if you wanted to do an analysis of metabolic expenditure-to-movement, would it be worth this kind of undertaking versus simply looking at line-crossings and comparing that to metabolic expenditure?

RA. I think that our sophistication has evolved to the point that we are getting different types of information from this more detailed and quantitative analysis. If we are just looking at a general activity, then crossing lines and that sort of thing is sufficient. But, in the null gravity situation, it would be interesting to look at more specific characteristics of movement patterns, because when you're asking sophisticated questions, this is the best type of system to use.

Basically, humans can only make three types of movement; plane movements, conical movements and rotary movements. And it's just a very basic question, it would be interesting under weightless conditions, if the distribution of these movement types for the various extremities would change. But I think you could ask a very, very precise type of question. But I look at it as the two not being mutually exclusive; they give you different types of information. So we do an overall gross analysis of activity patterns. But then we say, O.K., this is how much the organism moved, now, what types of movements did the organism make.

- JA. Let me take you over to rat pups a minute and sensory and motor task exploring. One of the things I thought you could offer me is the answer to the question of the test that you see as the most pertinent and most sensitive to damage and, therefore, one that might be the most sensitive to developmental perturbation.
- RA. Let's exclude anything related to feeding and sucking, at this point in time. I would say that the things I would really want to look at are essentially vestibular and cerebellar functions. Mainly because that's the kind of thing we're looking at with the premies. In early development, one could look at development of aversion reactions and their replacement with appropriate types of reactions. We have a sensory test battery that could be modified. In essence we devised a sequence of about 30 tasks per rat. The premie stuff is a little bit more difficult, mainly because you just can't pick them up and flop them around like you can with the rats. You have to be a little more tactful.
- JA. Are these different than your published tests?
- RA. Yes, we've dropped some of the old tests and added more complex type of sensory-motor testing.
- JA. Do you have write ups on those as well?
- RA. Well, I will have, since two of the students are using the battery for testing the malnourished animals. I have a proposal that would essentially go through the variety of tests. But none of them are extremely new or creative, they're modifications of our methods or Altman's. It's not really the test, it's how you use the test. We have some of these vestibular tests that animals should be able to perform equally no matter what their body weight is. And we have other vestibular tests that are going to be more tied to body weight, you know, interacting with their body weights and that sort of thing.
- JA. Could you tell me about a couple of those?
- RA. Well, basically we're looking at them in subtle types of ways, behaviors and geotaxic responses. We start doing our testing essentially on the day of birth and following through, but the responses start coming in around five or six days of age.
- JA. What responses are they?
- RA. The animal will orient differentially depending upon the angle of the ramp and the position in which you put them on the ramp, whether you put them on head down or head up. We're looking at righting-types of responses, and we're looking at the sequencing of how the body comes into the various righting responses. Most of these tests right now we're scoring from the time domain and scoring from whether they were accomplished in the past or not. And what we're going to use from this data then is the tasks which the animal shows the most interesting behavioral changes through and the tasks which they won't do any more, in depth movement analysis on them, find out which components of their behavior are really being disrupted.
- JA. What sort of video system do you have?

- RA. Right now we're using 16 mm high-speed film.
- JA. The reason I was asking was that if you happened to have one compatible with mine, I was thinking about sending you tapes of the simple vestibular tests that I'm planning on using.
- RA. What are you looking at?
- JA. One thing I'm doing is righting on surfaces; the do it simple policy.
- RA. I think the surface righting one is definitely the one you would want to go into with an in-depth analysis.
- JA. I think the opportunity for movement analysis is ripe, to look at exactly how they're righting. In addition, I test negative geotactic responses, using a tilt platform.
- RA. We have a tilt platform, too. What we wanted to take advantage of, because most of the vestibular tests are static, so what we do is we designed what we call our slip-and-fall platform. It's a platform sitting on a fulcrum, and you put the animal into the middle of it, so that any of the animal's movements will immediately cause the platform to tilt to one side or the other which provokes compensatory reactions.
- JA. Even in the zero and one-day-old?
- RA. No, they usually fall off until about four days. We're modifying angles and that sort of thing so that it can start earlier for longitudinal testing. But it's a nice developmental measure when they can handle it. The 15 degree angle we use now is a little bit too much.
- JA. I've been using 10 degrees and 5 degrees. I've been trying to find a threshold response.
- RA. Does your platform move when they move?
- JA. No, I designed one that moved: I was going to put them onto a level platform and then tilt it to a predetermined angle, at a known rate. When we started playing with it, I decided I was going in it too fast, getting into too many dimensions at once. To begin with, latencies to respond are age-related, plus the parameters of movement that I was generating confounded agewise comparisons, so I switched, just for simplicity at this point, to a fixed tilt for basic negative geotaxes.
- RA. That's more like our platform.
- JA. The only added feature of mine is that the animal is surrounded by mirrors, so that one can see the animal from above, from the front, and from the side, simultaneously. Hopefully people like you or others who are interested in more fine-grain analyses might find some other data in the video tapes of the animal turning uphill.

RA. What other tests are you looking at?

JA. The next vestibular test that we do is rotation. We get responses on Day 1. If you draw a line from the animal's snout down the middle of its head and spine, and then put it in the center of a turntable and give it a clockwise spin, its head deflects to the left and you can just measure the angle right off the video monitor. Some animals don't do it, but if they don't, they often show a compensatory response when you stop them. It's pretty.

RA. Are you getting any nystagmus?

JA. No. They tend to shift their head to one side and then leave it there. I realize I had never considered quantifying "strength" in rat pups, such as how strong their legs and back muscles are. Do you do any of that?

RA. We're not doing strength right now, what we're trying to do is get some measures of tone, it's a heavily-used clinical measurement.

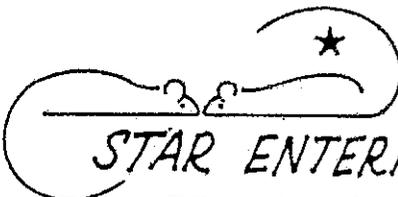
JA. How do you measure tone?

RA. That's the problem, there's no quantitative measure. I've spent a lot of time speaking with biomedical engineers. I told them what I thought would be a great way to quantify tone and so they are now working to see if they can develop a prototype instrument to quantify it. Right now, it's essentially in the clinical world and is basically handled through palpations and through resistance to passive movement. If you want, you can do an EMG. Tone is a measure that is used in all stroke patients. People think of it as a predictor of recovery: How tone changes within the first month following the stroke. So I'm very interested in developing an instrument that will quantify it, and working with the anatomists here, we've already developed the initial prototype. There's too much of the human element left in it. What I want to do is get rid of it as completely as possible, so that's why I have the biomedical engineers. I'm trying to get them to adapt it so that we can just look at tone, in animals, too.

JA. Do you conceive it as being something which palpates the muscles?

RA. Basically, yes. What we're using right now is a strain gage. What we're measuring is muscle springiness. We compress the muscle and then look at the ratio between how much compression and how much return there is to the muscle when we release the pressure.

D



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Jeffrey R. Alberts, Ph.D.
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INSIDE POOP ON THE RAT BREEDING EXPERIMENT, FLOWN ON COSMOS-1129

Interview with Dr. J. Richard Keefe (BioSpace, Inc.
& Case Western Reserve University Medical School).

Conducted by J. Alberts (Star Enterprises)

Dr. Keefe was the Principal Investigator on several embryological experiments flown on Cosmos-1129, and is a Principal Investigator on the upcoming Cosmos '83 flight (scheduled for October, 1983). His Soviet counterpart was Dr. Llyuba (Luba) Serova. The Project Managers were Dr. Eugene Ilyan for the U.S.S.R. and Dr. Ken Souza for the U.S.A.

Much of the information in this conversation has never been published as part of the Cosmos reports, due to its speculative nature and embarrassing implications. Nevertheless, it is important "lore" to recognize and accept as possibility. It is included here as background to some of the interpretive discussion of previous flights and as experiential data relevant to plans for future projects, including space station research.

- A. I would like you to discuss the previous attempt on Cosmos-1129 to have rats mate. The most useful information would be observations or data that were not included, or not stated accurately, in the published report of the 1129 project.
- K. I've intentionally forgotten what is in that report (joke).
- A. Let me ask you a question, just to get started. Was the rat compartment similar in size and structure, except for the partition, to the one we saw in Moscow?
- K. Yes, pretty close. As a matter of fact, I'm almost positive the one we saw was the exact dimensions. It may have been a little deeper, in that the waste handling may have been condensed down below, but the length and breadth were the same size.

A. Do you have any idea why the Soviets designed the rat compartment on the Cosmos-1129 with such height? If I was to make one change in the configuration of the animal compartment, I would have made it lower, so even if they're floating, they can't float away from each other. If a male mounted a female, he couldn't float off, the ceiling might hold him in place,

K. Also, I would give them something to hang onto.

A. Yes, that, too.

K. The five females and the two males were separated by that one compartment, and presumably, ground tests before flight simulation, all five females had become pregnant. And there were absolutely no difficulties. This was running it in a mock up of the space craft, not subjecting it to vibration and so forth, but to certainly a nominal profile as far as temperature and relative humidity and so forth, air handling were all concerned.

A. In the Synchronous controls for the Cosmos flight, did they spin these, did they give them hyper G to simulate lift off?

K. The problem is they couldn't do it simultaneously.

A. You mean that they did it five days later?

K. No, simultaneous from the stand point that they can't give them the accelerating G load at the same time that other things are happening: They can't step the G load, ramp it up and then ramp it down, at the same time giving them the re-entry shock. In other words, they have to take them off of one piece of equipment, and then put them on to another piece of equipment to get the re-entry shock. It's not a perfect match. But anyway, preflight, the story is (we've never seen any of the animals) that breeding was successful. I think they said that they ran it two or three times, and they had no difficulty with it.

A. Were the females that went up in Cosmos-1129 experienced breeders?

K. Again, we have never seen any offspring, nor have we gotten data, although we asked for it, and they told us that the data was available. The females were supposedly known, proven breeders that had delivered one litter before the flight. At recovery, the animals were weighed. We were not provided with that information until almost three months after the flight. As a matter of fact, we were provided with the information at the same time we had our summary meeting in California, because when I was doing the presentation for the summary, right afterwards, Ken Souza handed me the list of body weights.

So anyway, to go back to the Recovery, the animals were weighed, one of the problems was that we told them we wanted preflight weights and we wanted to see that weight profile as they went in. Because these were fairly young rats, we wanted to make sure the animals were healthy and growing normally. The data on each animal was just scattered all over the place. We questioned the Soviets and found that they used different balances to weigh the animals, and that the balances were not calibrated or accurate.

- A. You mean the data were scattered on different kinds of data sheets?
- K. No, the weights were scattered all over the place.
- A. The actual values were scattered?
- K. Yes, an animal would suddenly gain 50 grams, then lose 10 more, gain 50 grams, lose 60 grams, gain 30 grams . . . It turns out that the last body weights they recorded preflight were two days before launch; that, too, was done on yet another balance. That is one of the reasons why in the protocol that I have written for Cosmos '83, I specified the use of the identical balance. It's probably not going to do us any good, but I specified it anyway.

Back to the launch. The animals were weighed preflight, put on the paste diet, and so forth. We had done a simulation of our own, in which we took individual animals, put them in a mock cage we had built, and fed them paste diet, actually less quantity of paste diet than was ultimately fed to the animals. We fed them four times per day, once every six hours, divided the daily ration into four aliquots, and fed them once every six hours with this paste diet. The animals were absolutely ravenous. They consumed it as rapidly as it was put on the cage. Our animals were not handled, other than somebody coming up to tend them four times a day. They had water ad lib. The animals in our preflight simulation, were not handled at all during that 19-day period. They then were recovered, and weighed, and individually housed, but the paste diet was continued on the animals. We had three females and a male in one cage, and two females and a male in another cage. All five of the females conceived within two days of having access to each other. Based on a 21-day gestation period, they gave birth on day 22. We had no problems, normal births, well within the range for the animals (I've forgotten the precise numbers, but there were more than 8 per litter). No difficulty with the number of animals being produced.

- A. And this was the same Czechoslovakian stock used on the actual Cosmos-1129 flight?
- K. Right. So we had no difficulty, the animals were able to nurse, and there was normal growth of the pups, with one exception. One mother destroyed her young. We had reduced the litter to a common size, six or eight, and she seemed to take outrage and finished off the rest herself. So we lost one litter out of that group. I shouldn't say we had no other problems: The mothers continued to lose weight during the post natal period. It became obvious that one of the mothers in particular was in jeopardy. It finally dawned on me that the pups were eating the paste diet. The pups were competing with the mother for the paste diet. And as soon as I recognized that, I started feeding the mothers separately and returned them to the home cage after each feeding period. And that resolved the problem, but we did lose one mother. We managed to keep her litter alive. They were fine,

The loss of the mother rat, however, indicates that the paste diet was minimal in quantity and in nutrient level. We were giving 10 grams of paste diet four times a day, once every six hours, for a total of 40 grams per female per day. And during the flight, as I recall, the figure was 65 grams per female per day, again administered once every six hours automatically. So we had no reservations

in our minds about the ability of the paste diet to at least maintain the mother, keep her alive and have minimal impact on the development of the fetus. Now, by having minimal impact on the development of the fetus, I mean at least we got relative numbers, relative weights, although the weights of the offspring were lighter than the control weights, per pup basis. The question is whether or not protein malnutrition had an impact on the development of the nervous systems, something we looked at and we really don't know. But it is an area that would need to be looked at, but we did not do that. Now along comes Luba Serova postflight. The animals are recovered and weighed and she individually houses them, and continues to provide them paste diet, but the paste diet was provided maximum two times per day in whatever aliquot they felt like, from the stand point that if they thought about it twice a day, then they would give it to them in half the quantity, 30-35 grams at each time. And Luba "felt sorry for them", so she supplemented the diet with miscellaneous vegetables. We could barely believe that she would introduce this confound. They continued to weigh the animals and decided that one or two of the five were not pregnant.

A. Had she been smearing them prior to the flight?

K. No, she did not do any vaginal smears, to the best of my knowledge, she did no palpations, either. All she did was weigh and decided that she had at least three pregnancies and that these three she was going to let go to term. Now, we had encouraged sacrifice to look as soon as possible at Recovery because of the post flight stress and readaptation to 1-G. That's another question on top of the flight question. The Soviets, instead, decided, as is their usual case, to go for the whole ball of wax - go all the way to birth. We had no control over the decisions. There wasn't anything we could do about it. Then, ultimately, it must have been two weeks of post flight, she (Dr. Serova) finally decided that they weren't gaining weight very rapidly and maybe she had better open them up and look. She opened two of the animals, and immobilized the uteri and looked and counted implantation spots and yellow bodies. Serova decided that the animals had copulated, insemination had taken place, fertilization, implantation, placenta-tion, and that all of the animals were at the same developmental stage at the time of re-entry. She concluded that the re-entry stress, the shock, the banging . . . The 1129 not only had the initial clang and bang, but it got suspended in a tree and banged back and forth between tree trunks for a period of time before the army could get there to let it down. They had actually to cut down trees to get it down. The parachute did not separate, the parachute got hung up in the tree and the shroud lines kept it banging from tree to tree.

A. Do you know how long it was suspended and banged around?

K. No, it was anywhere from 30 minutes to an hour or longer, I mean it was not a five-minute period. So her argument was, the animals were all at the same stage, and they had all gone through this trauma, and this led to either abortion or absorption, although they saw no evidence of abortion in the compartment when they opened it up.

A. What was the grooming situation with the flighted rats? We've talked about this before, but . . .

K. This is something that has bothered me. They've (the Soviets) never taken pictures of it. I don't understand why they don't photograph these things when they open up the compartments. Liuba's description was that they looked very ratty in appearance, which isn't very scientific, but . . .

A. Did she say "ratty"?

K. No, she didn't. She was speaking Russian at the time, Galia, the interpreter, used the term ratty.

A. So you think that it was an expression meaning "scruffy" as opposed to "looking like a rat".

K. It seems to me there may be something about that in the physiologist. The other point in terms of scruffy looking, Grover Pitts (University of Virginia), who was the Principal Investigator responsible for the rat whole-body composition study on Cosmos-1129, also described the appearance as very poorly groomed.

Three of the females were mated subsequently and delivered quote, "normal litters". The flight males were mated with vivarium females, and sired quote, "normal litters". And the same was true with the Synchronous males, so we know that at least the males were capable of insemination.

Now, along the same lines using "identical protocol" was the Synchronous flight group (actually there was a five-day delay). What really blows everyone's minds is that there is this normal old mock-up space craft sitting there, the animals come out, they show similar weight profiles as the flight animals, and yet there are no pregnancies there! On the Synchronous flight! So again, Serova's argument is that everything went normal, copulation, fertilization, implantation, placentation, up to the same developmental stage, and then when they put it on shake, rattle and roll, that caused stress in the animals, and the animals failed, the conceptions failed.

A. But they did not observe the Synchronous animals to be sure they even copulated?

K. No, they did not. It's unfortunate, that would have been very easy to do. So that is the story. So far as Serova is concerned, the animals were pregnant. She was pushed for additional data, in terms of slides of the uteri which they were taken for from the animals on which they did laparotomies. The material was taken and supposedly we were going to be provided with copies of these. We have never seen them; we have continued to push for them. I talked to her about it in San Diego, and "oh, yes", it looked like there were absorption sites. I'll believe it when I see it. Until then, I've got to buy the fact that the animals were never impregnated, that they became acyclic, which is the story that Gene Illyan gave us.

A. But that's only his interpretation as well.

K. Gene Illyan gave me the simulation results afterwards. See, they couldn't figure out why the Synchronous animals didn't conceive, either, and therefore, it had to be some non-flight factor.

A. Had these animals been group housed prior to being put in the compartment with one another?

K. I don't remember. That was our recommendation, but I don't remember. We were looking for all kinds of strange little things of what went awry and so on. What we have done is taken an animal of known gestational age and subjected them in post flight simulation, subjected them to rotation, ramping up the G forces, tried to simulate the profile that the Soviets provided us with, the landing G force and so on and did that at Ames and had no problems with any of the animals. These animals were grouped in cages and so on, handled as identical as possible, except we knew exactly how old they were in terms of their conceptional age, so that we could expose 6, 7, 8, 9, 10, had we gotten there, periods that we chose. There were seven groups, each one at a selected gestational age, and all of them, with one exception, gave birth with no problems: We did not lose the conception.

We have reconstructed the flight in our minds. Illyan pointed out that the plexiglass air-handling cover, over the cage, may have permitted light leakage. There was on-board a lighting cycle project that had a light leak. Illyan's analysis is that the light leak caused the females to become acyclic and that, therefore, there was no way that they could conceive. We're going to be faced with the same problems this time (Cosmos '83) in terms of light if we're not careful, because we don't have light absorbant covers if Daryl (ef., Daryl Rassmussen - NASA, ARC engineer) is right. That's something if you get over there in August, you can see that, it would be very worthwhile to goade them. I think again, the Cosmos-1129 report, I think that what I said in there was that some "extraneous factor interacted", "non-flight factor", I think was the terminology I used.

A. It was the "execution" term that I remember.

K. At that time, I had gone back, looking at all kinds of things that can make animals become acyclic. One of the things that had rung a bell with me, and I went back into the literature and found it back in the 30's, was the idea of cleaning rats' noses with silver nitrate. The flight water for the animals is Moscow tap water, treated with silver nitrate!

A. Maybe we should bring back some Moscow water.

K. That was brought back at one point in time to analyze. Bill Heinrich can tell us about that. As I recall, there was nothing interesting spotted in that.

A. So, what you want to analyze is the flight water.

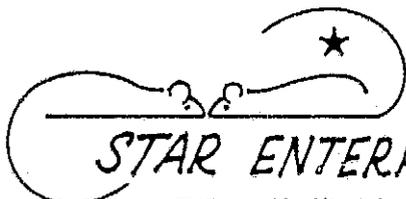
K. It would be very handy to have a sample of it, but as I recall, Ken requested a sample of the flight water and they told us it was not available, which may very well have been.

A. And what is it you think that Serova was looking at when she described the "yellow bodies"?

K. It wasn't until nearly a year later that we had the Final Results symposium in Budapest. It was at that meeting that I was ready to say all kinds of things. I had prepared a copy of what I was going to say to give to Galia who was doing

the simultaneous translation, I gave it to Galia in the morning and I was scheduled for the afternoon, and very shortly thereafter, Galia came running up saying how petrified that Luba was with what I was going to say and that she thought I ought to talk to Luba. I went to see Luba and she was accusing me of everything. You name it, I was doing it. This got into a real flap and ultimately took Gene Illyan and Ken Souza getting Luba and me together in a little room and hassling the whole thing out. It took about three hours of nightmarish debate over who was right and who was wrong, what is the scientific way to handle this versus what is the political, personal way to handle this. And the outcome was I pulled my punches and neutered my presentation.

At one point (during the formal discussion period), the Romanian coinvestigator with Luba, and I've forgotten her name, Luba finally turned to her and said "how do you say in English, yellow body", and the woman responded "corpora lutea", and that was when it dawned on me what she was saying, that she had spotted corpora lutea on the endometrial wall of the uterus. I had to explain to her that corpora lutea don't exist on the uterus, that they only exist in the ovary and then there are corpora lutea of several types. The lutea of ovulation, the lutea of pregnancy, that they would be significantly different between those two, and what she was probably looking at, and certainly the section would reveal it, was little pads of fat along with the blood vessels connected to the uterus. Those are rather common, you see a yellowish type of fat imbedded. All of this description until that time had been based upon the gross dissection. We had not seen a single microscopic slide of these uteri. I had seen two kodachromes, which had been taken at the time of laparotomy with a hand-held 35 mm camera, and the only way I could see these, this was in the institute at Planarnya. I got out a magnifying glass I was carrying and looked at it, held up to a window, with a magnifying glass, and I couldn't see anything. They were fuzzy, not in focus, or it had been shot with very slow shutter speed and there was a vibration, so that I could not make out anything on the surface. It was obviously a uterus. To say that it was slightly distended or swollen as you might find in a pseudo-pregnant animal, that's a possibility, but you really couldn't tell from that particular image. And that's one of the things that concerns me about this years project; whether or not the same thing is likely to happen, the animals come down and they're going to disappear. That's one of the reasons why I'm thrilled silly that you're going to be there and presumably going to have access to these animals.



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Jeffrey R. Alberts, Ph.D.
Research Director

STRESS AND PITUITARY-ADRENAL FUNCTION

An interview with Dr. Seymour Levine
(Professor of Psychiatry, Staford University Medical School)

Conducted by J. Alberts

Dr. Levine is one of the major national figures in the field of developmental psychobiology.

L. What has become apparent is that there are no quick and easy methods which can determine the state of an animal at a particular time. What you want is a variety of different configurations of events which are going to effect an animal and, in particular, effect the hormonal system. We're still dealing with the issue of nonspecificity of stress, which I don't really feel is realistic. There are sets of circumstances which will cause an animal to release cortisone, there are sets of circumstances which will cause an animal to release epinephrine, and other such sets of circumstances which will effect the reproductive system, and they're not all the same.

What is peculiar about space? In what ways does space differ from any other natural environment or any other environment? We're used to a gravity and, therefore, a set of stimulus elements, which are correspondingly determined. In space these stimulus situations differ, so that you maximize what I call the principle of uncertainty. Now to the extent that an organism could adapt to that . . . I don't really know. That's one of the real issues; whether or not this is an environment in which an organism can function. Now, if you want to determine whether an animal is stressed, then the question becomes, what the Hell do you look at? And you look at obviously dozens of different things such as blood, but you can't get blood . . .

A. Well, we might be able to. In the space station it would be conceivable that we could. People would be there on a continuous basis with the animals. Is it possible to tell NASA that there are one, or two, or half-a-dozen kinds of measures of stress: epinephrine, cortisone, or different urinary constituents, that are

responsive to different stressing agents on earth? Is there a profile of dependent variables? If so, and we found effects in space, and other animals that were centrifuged to 1-G didn't show these effects, then we could ask, are there ways that we could restructure the environment . . . ?

L. That's relatively straight forward. What kind of animals are you talking about - usually?

A. Rats. Although you could go on to squirrel monkeys in a moment if you wanted to.

L. Well, you could do it in squirrel monkeys . . .

A. I only added that because I think NASA is going to be flying some, not because I'm interested in them.

L. The squirrel monkey is a very responsive animal in terms of cortisone and other hormonal systems. You could look at a variety of measures in squirrel monkeys. But you're talking about rats. You're talking about in-flight or up at the space station. You would want to look at some of the gonadal hormones. At least testosterone seems to be very responsive to particular stimuli related to the female, so that you could determine whether or not they're responding normally under the conditions for which testosterone changes, that they would under circumstances that would not normally occur.

A. This last kind of testosterone response you mention, that's really quite pulsatile, isn't it? If you were to ask that kind of a question, you would present female stimuli, and then sample male blood discretely after that.

L. A lot of these things are episodic. The fact is that they also average. We get freaked out by the episodic phenomena, and clearly, if you're measuring testosterone, a single sample is not sufficient, but the fact of the matter is, if you have a level of testosterone, averaged over a period of time, you're going to see changes to stimuli. Those changes are going to be apparent over any pulsatile changes. There are pulsatile changes in cortisone, too, but the fact is that those pulsatile changes average with the time of the day. One of the obvious things you're going to want to look at is whether you create circadian rhythmicity in the subset. Animals normally show circadian rhythmicity. We know there are lots of controls to that rhythmicity. In addition to photoperiod, there's also feeding schedules. In a space environment, you will recreate light cycles, but you can't recreate all kinds of other things. Circadian rhythmicity is an important dimension. I would start with measures of the organism's rhythms, both adrenal and physiological.

A. When you measure circadian rhythmicity, what variables do you use? Do you have to do it from blood or could you . . . ?

L. Well, we know that cortisone is probably one of the most vigorous circadian rhythms, cortisone has a marked day-night relationship which is very clear, much clearer than most of the other hormones.

A. Can you do that from urine?

L. You probably could, I'm sure that you could do urinary corticosterone . . . , but I'm not sure that it can be done.

A. You don't do that in your lab, then?

L. It's a radioimmunoassay, no matter what you use a radioimmunoassay for, whether its urine or blood, is irrelevant. I don't know very many people who have used rat urinary hormones in general, primarily because you have to keep them in individual metabolism cages and so forth, so collecting urine is not anywhere as easy as collecting blood, actually.

A. So you'd want to look at circadian rhythmicity.

L. That would be almost one of the first things I would want to look at. We have well-established rules for what is . . . you could be able to tell, for example, whether or not there is any real dramatic change in basal condition by looking at circadian rhythms.

A. So under stressful conditions you would not expect to see the rhythmicity flatten-out or be disrupted?

The rhythmicity will change, either it will flatten out or it will, probably what will happen is your low levels will tend to be higher and your higher levels won't tend to modulate as much, so you see a shift in the peak relationships. Generally, levels are low in the morning, they'll be higher and they probably won't go as high in the afternoon.

A. If you put animals into a novel compartment or novel caging environment and vibrate it and had different noises than they are accustomed to, etc., would that be enough stress to shift these rhythms?

L. Oh sure, if I wanted to look at what changes would occur, novelty is one of the most profound things that alters these things we're studying now. The question is, I don't know how long it would take for animals to begin to start settling down.

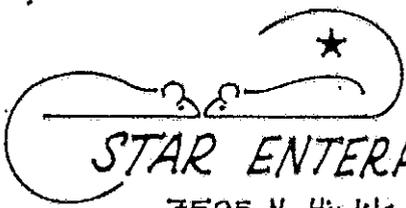
A. That was my next question. I know you can't measure . . .

L. I've never created an environment like that. If I just simply take them from one cage and put them in another cage, I would expect them to settle down within a couple of days. But I just don't know about this situation, it's so very alien. What would be nice to see is what the second and third generation would start doing, assuming that you could get a second and third generation. But that's a different question, whether or not you can get a second or third generation. There are a whole set of developmental issues of reproductive endocrinology that you might want to ask as to whether they would develop normally or not.

A. And there what would you be looking at?

- L. Don't ask me, because I'm not into the adrenal system. I have spent a good part of the early part of my career looking at the development of adrenal systems, and I know what they look like, in terms of what normal development occurred, and I know what sort of things could create abnormal development, at least nutritional things that create abnormal development, yet I don't know about animals in this environment. So the question is, assuming that they do develop and then you get them to reproduce and develop offspring. There's a whole pattern of when babies begin to mature, when they begin to show appropriate responses to stimuli, what they look like postnatally.
- A. And again, you would consider it valuable to ask those kinds of questions with the adrenal measures?
- L. I think they are very interesting questions, given that you are born into an environment that is alien to the species, which would not be alien to you. To what extent do you begin to adapt normally to that particular environment.
- A. How early can you use your hormonal measures?
- L. Oh, one or two days of age. Actually, we've done steroid samples fetally. You could certainly do them immediately postnatally.
- A. And those are sensitive to maternal stress?
- L. We don't know, because I haven't really done maternal stress studies. I don't know if anybody has done maternal stress studies, looking at cortisone. Most prenatal stress studies, and they are doing this with humans right now, are more involved in pregnancy outcome measures, with the exception of the Ingeborg Ward stuff, which looks down the line. That's a peculiar stress.
- A. Heat-plus-light?
- L. Heat-plus-light, yes. That's a very strange phenomenon. There is, however, a very important age-dependent aspect to the pituitary-adrenal system. We have found that with aversion learning in the adult - if you block that system, you don't get taste-aversion learning. In the case of the infant, the question is when it occurs and when it doesn't occur and under what circumstances it occurs may be related to how much, the capacity for that system to be activated.
- A. Are there any general statements you can make about that?
- L. Well, what you find, generally speaking, is that if you look at the developmental stage of the animal, the early ages are very active and the animal will tend to show relatively high levels, will be able to show certain kinds of responses to some stimuli. But really, the interesting down period is from roughly five or six days up to about 12, 13, 14 days. Even if you slug that system with huge amounts of ACTH, it doesn't work. You can't get it to respond to any stimulus at all.
- A. Until when?

- L. That lasts until about 13 or 14 days, and then the system starts taking on adult characteristics. It is not really fully adult until 29 or 30 days of age. There are a lot of nuances, but it generally will respond.
- A. You have a similar notion of buffering. You and Smotherman talked about it in regard to the mother, where it results from suckling. Does this suggest that if lactating animals were kept with their infants, they would enjoy some kind of buffering? Might this be applicable in space?
- L. The lactating female, it is interesting, because she's less responsive to generalized stimuli and more responsive to the pups.
- A. Does she need pup stimulation to get into that state or is there a pregnancy effect?
- L. There is a pregnancy effect in terms of the buffering. . . But the fact of the matter is, Jeff, that I could think of a million things that you could do.
- A. It sounds as though I could recommend that pituitary-adrenal measures be included in a protocol.
- L. I would think you would certainly want to do that and you would certainly want to look at what happens to the whole toning of the system, whether or not the animal becomes more responsive or less responsive, whether or not they maintain normal rhythmic relationships, whether or not the developmental patterns of this animal are essentially the same. If you're really asking what changes, the cortisol system is not a rampant one, it seems to be particularly responsive to some kinds of psychological myths. And it's also a very critical system in terms of the immune responses. One of its major functions is a suppressant, so you really don't want that system triggering at very high levels if you want to maintain healthy animals.
- A. Another thing I was going to say is that another wise thing NASA could do would be to think ahead and be sure that they've got the normative data from earth experiments.
- L. Well, there's so much of that around. If you take your blood samples from rats, it's really not very hard.
- A. But there may not be. For instance, you were talking about moving animals from one cage to another and so forth. . . that's the kind of situation where there are suggestive bits of data around, but it would be useful for them to be conducted systematically.
- L. Well, there is suggested bits of data around where they've been moving an animal from one cage to another, but they are generally short-term effects, nobody has really looked at any long-term consequences.
- A. That's the kind of normative data they really should invest in.



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Jeffrey R. Alberts, Ph.D.
Research Director

PERINATAL STRESS AND ITS EFFECTS ON OFFSPRING AND MOTHER

An interview with Drs. Ingeborg Ward and O. Byron Ward,
developmental neuroendocrinologists (Villanova University)

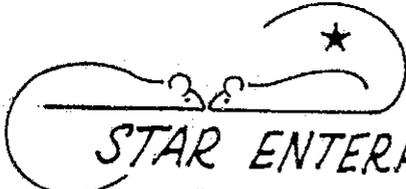
Conducted by J. Alberts

- IW. Stress does play havoc to the hormonal system. You have, on the other hand, various adaptation factors to contend with. In fact in the hormonal system, change is a function of timing. And if you're dealing with developing organs, then the impact depends upon the point in time that the changes occur. Consequently, it's difficult to predict what you're going to get.
- A. So would you recommend that the best time to do an ano-genital (AG) distance measurement would be on day zero or day one, immediately post partum?
- BW. Yes.
- A. Well, that is probably quite favorable since there is very little else I want to do immediately except examine the animals.
- BW. You'll get body weights at the same time?
- A. Yes.
- BW. The pregnant animals are going to be group housed?
- A. Right. They float around in their cage. Along the wall there are disks that are spring mounted, flush to the wall. The animals learn to press in the disk with their snouts and from it can lap up this paste diet that sort of exudes.
- BW. Are they trained before they go up?
- A. These aspects of the experiment are sometimes infuriating, at least frustrating. They're not habituating the animals to one another or to the compartment.
- IW. Are they recording the amount of intake?
- A. No, they can't, because all the animals are eating from the same feeders. So all we'll have is the body weight of the animals at the time of lift-off and the body weights when they return, and there may be a measure of total food gone. But I don't know if they even know to what extent there's spillage. They certainly won't know for individual cases.

- IW. I guess that would become problematic then, because malnutrition in and of itself would be probably as stressful as space, if not worse.
- A. All we'll have is the body weights of the animals when they take off and the body weights of the animals in the two control groups.
- BW. One of the things that I would expect is a delayed parturition. And you won't know that unless you observe the mating. We mate our rats at a particular time of day, let a male ejaculate twice into the female, and time the first ejaculation. People that just look for the presence of a vaginal plug or something really don't come close to being able to time the breeding. And I think one very likely thing that you might see, as there being some evidence of the animals having been under some stress, is a delay of 24 hours or longer in parturition.
- A. Is your hit-rate good, using your direct observation method?
- BW. Oh, yes, we miss very, very rarely.
- IW. But even then, you get an occasional male that's copulatory ability is fine, but females never get pregnant. These animals may have no sperm. So it's a good idea to observe and to do a lavage. Even if you find a plug, you can't be sure that there are any sperm.
- A. I would like you to talk about experiments and procedures for a space station, anything that has relevance to designing a laboratory in a space station, including how much room and what type of apparatus should be available.
- IW. We've just published a bunch of studies on attempting to characterize the physiological mechanisms and its relevance to stress symptoms. Among the things we have looked at are various enzyme systems that are involved in sexual differentiation, for example the aromatase system in the brain. We find that brain aromatase levels are down in fetuses and in mothers that are stressed. Now whether that would work in space is another question. We can send you a reprint of that. Byron was Dr. White's assistant, and out of this we also published a paper on fetal enzymes. They show altered patterns of stored activity, markedly altered and directly correlated when the blood level of testosterone is high. So we're beginning to feel that we're figuring out the link between specific mechanisms and differentiation. But all of these would involve getting hold of, exhibits of particular components of these at a particular point in time.
- A. In the space station, such things might be feasible.
- BW. True, that might be feasible.
- A. So in a space station, to what extent could a non-endocrinologist be trained to do the necessary removal and fixation. Is it possible to train someone to do the necessary techniques?

- IW. It should be. We do a great deal of collaborative work. We often prepare animals and various technicians come from other labs to take the tissues away. For this testing, they usually do the endocrinology on the same day the tissue is removed.
- BW. We've got a possibility now of up to 48 hours delay on that.
- IW. But with the space station you couldn't get the stuff down in 48 hours.
- A. A space station may offer many options. For an experiment of the sort that Byron just described, and for the kind that you have in mind, Inge, it might be possible to conduct the full experiment in a station, including surgery at null gravity. It's possible that there would be portions of the station with a live-in centrifuge for people who were there, workers, at something like 1-G.
- IW. At the present time our collaborator extracts the tissue out of the living fetus and then we freeze it. The question is whether you can do it the other way around, freeze the brain, bring it down, and then extract the portion. I think those are all the technical questions, with them, whether or not you want to change the procedure. . .
- A. What about maternal, have you ever looked at how individual mothers reacted to the stressors and then correlate that with the responses that the offspring are showing?
- IW. What we attempted to do one time was simply to record body temperature changes of stressed mothers to the extent to which we got alterations in the feeding group. And I'm afraid that that didn't work out too well, we have to redo it. We were really surprised, there was enormous variability from animal to animal, within animals.
- A. What range, a couple degrees?
- IW. Yes, in the changes to the supposedly standard stressed animals.
- A. . . . And mice, as far as comparing rats and mice, are mice better subjects for this work?
- IW. This has never been done with mice, at least our particular experiments have only been done with rats.
- BW. It's been reported, though, at one of the meetings we just returned from, that the phenomenon does exist in mice.
- A. In females as well as males?
- IW. That data hasn't been published or really been presented yet, so we can't really evaluate whether or not it is valid.
- A. One of the things I'll want to do is evaluate the choice of the rat versus other organisms, such as mice. The fact is that at the current time in published literature, there's far more information available with rats.

- IW. As far as sexual behavior is concerned, we don't know that much yet about just what point in perinatal development the crucial events occur in other species or what their stress limits might be. . . I think the rat is definitely the one to go with, there are many strains.
- A. My experience with mice is that the strains are probably even exacerbated with the inbreeding they've been subjected to,
- IW. I think that's one other thing that, at least in our experiences with the stress cages, has been that some of the mothers simply dropped dead, they do that relatively early on.
- A. This is the restraint-plus-heat?
- IW. That's correct. If we're going to lose the animals, we tend to lose them on the second day. And I guess one of the things you learn out of that is that the survivors are a different breed from the ones that don't make it. So you already have a selection factor in terms of the animals that you're going to have at the end of the study; they're the offspring of those mothers that could tolerate the stress.
- A. Have you actually done just the restraint and just the heat and found no appreciable effects and that you need both of those?
- IW. We haven't done it, I think Judy Setern tried that and she got no effects with anything. All three of our treatments together got nothing, and she tried just one or two of them at a time and that didn't work either. So she never got the syndrome no matter what she did. . . . Do you think weightlessness is a stress?
- A. One of the reasons is that I think the animals will find the novelty to be stressful. Also, rotation and the kind of vestibular manipulations that I associate with the experience are things that have been shown to produce food aversion and so forth, so again, I think it's the kind of thing that they may, in the looser sense of the term, find stressful.
- IW. I take it that there are no studies that have been done in this country where they've used weightlessness and they took blood samples and demonstrated that corticoids were up or down.
- A. Well, as far as I know not in rats, and I would find it hard to believe that they haven't done that in primates, and I don't know the data if it has been done.
- IW. So essentially, if one were really impressed by specific questions, there isn't any way of demonstrating them by accepting criteria on these animals caused through stress.



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An interview with Dr. Timothy Schallert
(University of Texas at Austin): Vestibular Specialist

Conducted by J. Alberts

S. I've been thinking in a number of different ways the idea of testing animals before, during, and after flight in space. The idea that space has some sort of consequence or series of consequences on the animal's vestibular function. I've been thinking of what I might do to test something like neurological deficits and on top of that, I've been thinking of a prespace protective procedure, and in-space protective procedures. Before the animal goes into space, what could you do to a group of animals, how could you treat them to allow them to better able to adapt to gravity and then in space, what could you do while the animals are in space in order to permit them to have fewer negative consequences on the space flight. And then, of course, within that you'd look at, in your untreated groups, the effect of space, per se. The question is, what kind of tests would one look at.

The immediate thought is a test of a vestibular and related systems, sisters to vestibular function, sensi-motor functions, but the vestibular functions themselves. You think in terms of swimming tasks and righting. Air-righting versus other kinds of righting. Responses to linear and non-linear acceleration.

A. I'm interested that you're bringing this up, because I've gotten to the same place in my thinking: What you're calling preflight protection issues. There's a fellow at Ames, named Jiro Oyama, who has conducted a lot of centrifuge studies. He looks at adaptation to hyper-G and then re-adaptation to a lower, 1-G force. I wonder if having animals undergo experiences like that, so that they have experiences going from a higher G to a lower G, if that would then enable them to make better adaptation from a 1-G to a zero-G.

S. Just to be able to shift. That would be an extremely interesting situation to have an animal sort of born and raised in that situation, you'd have the equivalent of a blinded animal.

A. Well, it's a deafferentation; that's the way it's been treated.

- S. And one in which you can get it back, you don't lose the sensory organ, you can look at it later. You can look at the neurology, you can look at the cells in the vestibular system and see that. And also you could do it in adulthood versus infancy, which would be extremely interesting. There are so many different experiments one could do!
- A. I think that's what this would be about, sort of driving that message home. There are lots and lots of wonderful experiments.
- S. You may be able to protect some of those neural systems. I've been wondering what an animal would be going through with respect to gravity in the water. They're still experiencing gravity, but they're missing some other parts. An animal in water assumes postures much like those of an animal in free fall. . . . If an animal has had a lot of experience swimming, what would that be like in space. Also as a possible test later, their ability to swim might be interesting.
- A. What happens with Parkinson's-types in water?
- S. That was a very interesting experiment. One thing I have always wanted to do is give an animal haloperidol and put him in space, because an haloperidol-treated animal in the water just sits still. If you put a normal animal upside down and it rights, if you put a haloperidol animal in warm water and it grasps something, it can cling, you can turn it upside down. I don't know if you remember, I did an experiment which showed that haloperidol-treated animals right perfectly well in an air-righting test. They're like a righting machine, all their locomotion is turned off, their scanning is turned off, lots of different systems are turned off; the vestibular system remains, so it's like a little righting machine, dropped in the air, perfect righting. Put it on the ground, it gets contact righting all of those systems. If you put it on a smooth surface and tilt the surface, and you get all of those responses to linear acceleration and so forth. But if you drop it in the air upside down and it has something to cling to, it lands on its back! Righting is completely shut off. The Clinging response inhibits righting; it's a natural inhibition. Think of a squirrel falling and grabbing a tree. Well in space, if you gave, . . . I don't know what the haloperidol-treated animals do. A normal animal would be contaminated by all kinds of things, it would be struggling, trying to move and scan, I'm not sure what it would do, it would be interesting. A haloperidol treated animal, now you have an animal that probably otherwise would have no cause to move unless you challenged its labyrinthine system or some other vestibular-related system. Unless you challenge that animal, the equilibrium of the animal, the stability of the animal, it's going to stay still. So what would it do in the air without gravity? Would it be continuously trying to right? I can see this animal showing a barrel rotation or something. It would never satisfy its receptors. What could you do to shut it off? Give it something to cling to and that might stop it. It would cling and then it wouldn't have to try to right. I don't know what it would do.
- A. As far as I know, no one knows what the few rats that have been in space have been doing, normally.

S. I would say that the preparation of choice of really studying it in isolation would be the dopamine-depleted animal. Of course you would look at normal animals as well, but the dopamine-depleted animal would be a little machine up there that you could test to see what happens, and then you could see what it does to angular acceleration, see what kinds of postures it has, and so on. There's a real good rationale for it.

A. How long does the haloperidol treatment last?

S. It depends. If given via a minipump, it could go on almost indefinitely. One shot would last three hours. You could deplete the animal with dihydroxydopamine and it would go up and just stay that way; it would be permanent.

A. But, do such dopamine animals feed themselves?

S. No, not really, you'd have to intervene.

A. Before we discuss neurological deficits, what kind of observation methods do you think would be the best for preliminary assessments of animals at null gravity?

S. Are we talking about in space observations or the after space observations?

A. Let's say in space first.

S. One of the first things I would look at would be the postures of the animals while they're immobile and then during, on film.

A. Would you have to film, and would the compartment have to be specially designed, in other words with mirrors, or just a single point of the camera.

S. You'd probably want mirrors if you have more than one animal; you could lose a lot of information otherwise. It's not much more to have mirrors, that would be good.

A. And would you use a movement notation system?

S. It would be ideal for movement notation in the sense that you could describe it pretty accurately. There are lots of different systems developed in the field of motor systems of animals and in the, . . . physical education has a number of different notations, it wouldn't have to be ethical-Bockman, but it could be, you definitely want to quantify the angle of the lens in different situations, I would think.

A. Is a single camera sufficient for that?

S. You'd want a movie camera,

A. As opposed to video?

S. Video cameras these days are pretty good, color video cameras give you quite a bit of information. I've been able to do pretty good analyses with videotape; it's handier. That's something I just wouldn't be able to tell you, I don't know that it would make that big a difference, You'd want something that's clear,

A. But a single viewpoint is sufficient?

S. Well, if you have a mirror, yes, you'd probably be able to get it.

A. But more than one camera would be desirable?

S. I'd have to see the apparatus, if I saw the apparatus, it's probably like a small box, right?

A. Well, for the space station, that's really an issue. NASA has no designs whatsoever and that was by plan, so that when scientists come in and say what they need. . .

S. I haven't thought this through. Of course I have a swimming task, I have a number of ways of rating swimming behavior, and one of the first things that goes when there are vestibular deficits is the ability to swim properly and orient in water and navigate in water. So I've modified a navigation task that's becoming popular now. Essentially it's finding a hidden platform in the water. But you can look not only at the way the animal finds the platform, or the ability of the animal to use visual cues in order to orient itself in space, but you could also look at heading error and distance travelled and compare that to how long it takes the animal, so you get sort of an idea of the actual ability of the animal to swim versus its ability to navigate and orient itself. These are all computer-quantified. After about four or five trials, there is almost no variability. It's incredible to see it. It's one of the best tasks I think I've come across and I've got it so thoroughly down pat that we're having a lot of success with it. When the animal gets back on earth, it would be real easy to film these animals righting and look at the initiation of righting as well as the speed of righting, and the accuracy of it, and so on.

You could measure the thrust in a number of different ways, the actual strength of the antigravity response the animal might make. When the animal is dropped, even before it begins to right, if you just have the animal sitting on a surface and you drop the entire surface, the animal thrusts immediately and this is the typical response to a debt acceleration. This sort of thing could be measured. If there's a deficit in an animal raised in zero-G to that response, you could measure it. This response, by the way, comes in at I think at about 15 days, but you could measure . . . if you put an object, a glass or anything onto a piece of wood and drop it, the wood stays glued to the platform, because they, of course as Gayle Lehrer will show you, they respond at the same speed no matter how light it is, it gets a lot of friction from the air. If you put a rat on the board, it separates immediately from the board and you can measure the distance between the board, the platform, and the animal, and that is something of a measure of the strength.

A. Is that because it is pushing the board away from itself?

- S. Yes, it's pushing it so that the board accelerates faster than the animal. If you put an anesthetized animal on it, it stays glued to the board, so there is something that the animal is doing actively, and these are the kinds of things one could easily measure, you could get a quick estimate of it, and I would think that that sort of thing you could use to measure the ability of an animal to right after, you know an adult animal that's had experience at zero-G or an animal raised in it and so on. That type of thing.

After working with an animal a while, even after a few days of seeing an animal that's been subjected for a long time to that experience, there could easily be tests you could come up with. You could just run through the battery. I'm a big believer in the battery. There are going to be multiple deficits, there are going to be some deficits that are due to something else and then that sort of indirectly suggests that they have such and such a deficit, but you could pick it up by giving them a whole series of tests. And you can also develop new tests, there are lots of new kinds that could go. You need to work with them a while and be flexible, but these are the kinds of things you might start out looking for to see if there is anything of a vestibular sense. Run them through a battery of vestibular experiments. Put them under water, upside down, and see if they immediately come to the surface to measure latency to come to the surface before and after the space situation. An animal with impaired labyrinthine function, even unilaterally, will have real trouble. You'd do it in a dark room. You have to remove vision because the vision can take over the function. That's one thing you're always going to be dealing with, the ability to respond to the environment, and there are multiple ways of solving these problems. You probably always want to send up a group of blind animals because with vision, you may find that an animal comes down with no neurological deficit, but they have been able to compensate through visual control of some kind. This is really true with people and animals; one of the first things you do if you want to test vestibular functions in people is to put a blindfold around them and then you see what they can do in that situation. You see some tremendous deficits come out in people with vestibular dysfunction that you wouldn't find if they can use their sight. If there is a vestibular dysfunction, the fact that the animals can compensate visually, you might want to train them in their ability to use their visual system. You might find that if they've had that training, when they get in space, whatever it is that lack of vestibular function does to an organism, it may be not directly linked, it might be on a direct line to that vestibular loss, but it may be that it is an orientation loss, so that if you could provide lots and lots of visual cues to show them which way is up . . .

- A. So have them in a striped environment preflight and then give them a striped.
- S. Yes, give them cues and put them in situations. Pilots are able to do things much better and are less likely to be disoriented than other people when they're upside down and so on cause they've learned in that situation.
- A. What about bandaging them, do you think that would have any beneficial effect?

- S. The bandaging may provide the contact, I published a paper on that response in which I related the bandage to the clinging response. The bandage provides support for the head. It simulates resting the head on a surface. The animal acts as though it is head down on something, and it does the same thing as clinging. If an animal is bandaged, it doesn't right in space, if an animal clings, it doesn't right when free falling. When the head, in particular, is given this support, it falls back as though it no longer needs to keep its head erect. And it could be that while the people are sleeping, they are unable to use the vestibular clinging cues, or animals the same way. When they go into that phase, it may be good to provide a bandaged kind of environment, a pressure kind of environment. Of course, you may be able to get that with acceleration, too, or with centrifuging as well. So that is something that could be done, that sort of support.
- A. If that were true, it might be a way of doing, if experiments were necessary, to do a sorting out between physiological effects of null gravity and stressful effects of null gravity are mediated by things that could be ameliorated or lessened with a prophylactic like a bandage or something, so that the bones are still subjected to whatever those conditions are, but not to say the adrenal responses of stress.
- S. It could be that, there are so many things it could be. I would think a lot of it would have to do with the muscles and standing still, there's got to be some atrophy, but that acceleration component might be really important for the animals. But if it isn't, that would really be revealing. If you spin these animals and find that the bones still change, then you start looking for other components.
- A. What about experimental labyrinthectomies: Are they any good?
- S. I've thought about that, there are nice ways of doing labyrinthectomies in animals.
- A. What if you wanted to maximize mating behavior in space?
- S. That is something, well, I hadn't put those together, but that's a very good idea.
- A. Find out what kind of an environment you'd need to have a labyrinthectomized pair of animals mating in the dark. So you'd train them, you'd use that preparation to look for shapes and contours and surfaces of environments that would allow them to mate.
- S. That would be a good idea in terms of getting animals to mate.



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DEVELOPMENT AND REPRODUCTION IN SPACE AND RELEVANT ASPECTS OF SL-4.

Interview with Dr. Chris Schatte, Project Scientist, NASA - Ames Research Center.

Conducted by J. Alberts (Star Enterprises).

Dr. Schatte is Project Scientist at NASA-ARC. He is, among his other tasks, responsible for many of the life science experimtns on SL-3.

- A. Why are you interested in reproduction and development in space?
- S. I think that more than anything else that we could do right now, breeding an animal and watching it grow in space will give us insight into some basic biological questions of ontogenesis. How would we look if we had grown up in less than 1-G? In this other environment, what might be our shape, bone density, the way we grow? I think over several generations, our basic form, such as having our arms and legs at opposite ends of the trunk, might be different. If you look at the whole space boat program, concerning why we're flying animals into space, the most political purposes concern human medical problems. We've got problems that must be resolved. But when you come right down to it, the question becomes will there be any reason to do life sciences research in space once human medical problems are resolved. And the answer is definitely yes.
- A. So you're interested in using space as a tool to learn more about life on earth?
- S. Right, and not so much the problem-oriented tasks, but why we do the things we do, why we are the way we are. And weightlessness is certainly the biggest factor in space that is a change from the earth environment. In addition, you've got geomagnetic radiation. I happen to have a strong interest in rhythms, and of course, you know one of the theories is that geomagnetic radiation is a kind of zeitgeber; it controls some of the rhythms, according to so-called exogenous theory.

Well, you get away from the earth and you can get rid of some of that. This may not be an identified medical problem right now, but it would be fascinating to study. The answer might tell us why we sleep as much as we do and why we sleep when we do, and so on. So anyway, that's the reason I'm for it. In addition, I think that that would grab the minds of the public more than anything else. They'll read that we've cured space motion sickness. Well, they're going to say, that's terrific and how's the weather. They're not interested in space motion sickness. But, if you say here's what an animal would look like, here's why we look the way we do, here's what an animal running on the moon might look like and so forth, that would be a real grabber.

- A. You call it politics, I call it culture, I think this aspect is one of the most exciting, one of the reasons that is NASA important. I think it's an exciting part of our culture.
- S. The same people that will go to 2001 Space Odessey, will see E.T. or go see Close Encounters of the Third Kind are going to be fascinated by that kind of analogy. I think there is a lot of utility in doing that, too, but NASA's life sciences area is dominated by the medical aspects right now. Overall, of course, engineering is the biggest, I think the fascinating things in life sciences are neither one, neither medical nor engineering.
- A. Let me ask you two questions. One was when you talked about zeitgebers. This is going back to the evaluation of the Cosmos flight. As you know, the animals didn't come down pregnant, and, as you might know, the so-called synchronous controls that were run on earth also failed to get pregnant. I've been going through the protocol to show what things might have been attended to more carefully and so forth. I've noticed that the feeding schedules were very peculiar. The reason they used the peculiar feeding schedule was because they used a paste diet to feed the rats on the Cosmos mission. Apparently, it dries into an extraordinarily hard and difficult-to-digest mass. Their solution was to present at discrete intervals. They anticipate the average consumption of a group of rats, divide that by four, and then present one-fourth of each daily ration at six hour intervals.
- S. At even six hour intervals?
- A. Yes.
- S. That doesn't make any sense at all.
- A. If they have a reasonable day-night cycle (they were using a 12-12 cycle on the flight) the rats would eat most of their food during the dark hours. Dick Keefe believes that there may have been light leakage, so that the animals may have been on continuous illumination. They may have been flown without an illumination cycle, as a zeitgeber. Many years ago, Curt Richter showed that food presentation can also be an effective zeitgeber, but the distributed presentations of food may have obviated that time cue as well. These conditions may have put the females into continuous estrus, in which case they are anovulatory.
- S. And they have no way of checking it either, do they?

- A. Absolutely not. But it is an interesting problem. It's remarkable to see how something like the characteristics of the food might actually shake the rock under the experiment. Now I am curious about the food that might be used on Space Labs.
- S. The way the food is set up on SL-4 is a lot better. It is essentially ad lib, and they can cycle the way they want. I think also the lighting, the design on the RAHF, is such that there isn't going to be leakage. It is optically tight, and there will be the right cycle for them.
- A. Will it be set up and instrumented in such a way that the food intake will actually be monitored?
- S. Oh yes, it's all definitely recorded. They have on the water system, a set of solenoid valves that are very sensitive to pressure, and when they lick, it's just a very slight positive pressure, and whenever they lick, water will flow only when they lick. And the solenoid puts out in very discrete intervals, I think it is a milliliter at a time or something like that, but it can do it as fast as they can possibly drink, it registers each milliliter. And the food is the same way, it has a little, it's a bar that goes along a track and there's a little microswitch that rolls over and over as the bar moves. It will record down to less than a tenth of a gram. So it not only will get food consumption, but will get the rhythm of it.
- A. Another general question. Has anyone done standard metabolic measures, oxygen consumption, CO₂ production on animals at null gravity?
- S. In space? No, as a matter of fact before I came here, I had a NASA grant to do exactly that, and I submitted a proposal for this. Unfortunately, it didn't make it. The reason that it hasn't been done so far is that NASA priorities are related to biomedical problems. Nevertheless, with the negative calcium balance resulting in bone demarrowization, and also the negative nitrogen balance with muscle atrophy and deterioration, we are going to have to do some very intricate balance studies. You have to essentially do a caloric balance, too, which gets into oxygen consumption and more. But for the rats, unfortunately, there is probably a major flaw within their containers, which were not designed to be air tight, so they are not going to be done in the space lab. One of our projects now, we are just meeting to cut a deal with the French, they're going to build some hardware to fly large primates. They are building modules for monkeys which will allow those kinds of studies to be done. Also, NASA is looking at the possibility of building a mid-deck facility which would fly not in the Space Lab, but on the orbiter. So it could go up any time the orbiter went up as long as there was room, which will put a single monkey. This is really tricky, statistically, but over a period of several flights, you could accumulate data.



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An interview with Dr. Eugene Sackett (University of Washington)
Specialist in Primate development and behavior

Conducted by J. Alberts

- S. There is a special breeding population of female pigtail monkeys that are all multiparous. In this set they have each had at least four or five prior pregnancy outcomes, and for the ones that I would be interested in for a pilot study here, they would be ones that have excellent pregnancy outcomes, never had a bad one. We also have menstrual cycle data on them as well as data on their parturitions, three prior parturitions for some of them. This is a videotape data, so we also know about their sleep-wake cycle in the month before they deliver. We also have a quantitative observational coding system from the video, describing behavioral changes as they approach delivery. The sample size for some of the measures on that is up to 180 animals. On another 40 or so, we have data on post delivery behavior with the newborns. We not only have normative data for the species on parturition in a single cage, these are all single-cage parturitions. We would even have data on either 2 or 3 of their prior parturitions. The basic problem is straight forward: Under conditions of weightlessness, what happens to the course of parturition and labor and delivery and also post-delivery responses to the newborn and the newborn's own behavior, which is essentially nothing except climbing on the mother and hanging on them.
- A. These are factors dependent to some extent on their orientation and the proprioceptive cues?
- S. Somewhat, this is one of the reasons, it is really interesting, of course, the negative geotrophic response to climb up the mother or to climb up anything, and what they would do under space conditions and how the mother would compensate if they didn't is an interesting issue in and of itself, but the main issue here, I think, is what happens to the course of parturition, itself. We also have some physiological data, mostly heart rate and temperature. Now temperature is a fascinating thing with these monkeys because approximately half an hour before delivery, they have a huge temperature drop, in some cases up to two degrees Centigrade.

A. Measured where?

S. Measured with a transmitter that is subdermal under the neck skin, a scapula transmitter. They have the giant temperature drop and then within about 15 minutes before the actual delivery, it goes back up again and overshoots, and that seems to be very consistent, the magnitude differs somewhat between females, but the temperal aspects are about the same through all the ones we have studied.

A. What time of day do they deliver?

S. Night. Almost all of them are between 8:00 at night and 1:00 in the morning.

A. One of the Principal Investigators on the Cosmos project is a man named Frank Salzman, from Binghamton. Do you know him?

S. The name sounds familiar.

A. He works with Rhesus macaques, and his project and most of his research is on circadian rhythms in Rhesus, and in particular on temperature rhythms. He does a little work on dissociation of activity and temperature and a few other things, but temperature rhymicity is one of the four experiments on the Soviet flight that he is doing.

S. Well, one of the interesting things about this parturition business is one of the simplist kinds of data for us to take, which we have taken, this is the one where we have N's of around 180, is sleep-wakefulness states as measured once every half hour, 24 hours a day, from observations in the month before, approximately the month before delivery. One of the things that we found is that you cannot predict the day of delivery from changes in diurnal cyclicity until the day of delivery. Even the day before there is just no variation from what it has been before that.

A. So you could be interested in looking at the parturitions and post parturition behaviors of animals that were flighted during pregnancy?

S. Oh, yes. The basic idea would be to get these animals like we have been doing all along, essentially about a month before delivery and start taking data on them under the basic housing conditions, whatever this would be in the actual flight and in orbit, starting about a month before the expected parturition, and of course that being when the flight is, and that be all the baseline data that we can under the basic housing conditions for somewhere two to three weeks before the actual flight.

A. Are you especially well equipped to analyze behavioral data?

S. Well, we have spent seven or eight years now working out coding systems for this.

A. What do you measure?

- S. In the monkeys, now, there's a home cage system that deals mainly with activity, and there is a sleep-wakefulness coding scheme that generates diurnal cyclicality data. From these, it is possible to generate the most beautiful diurnal cycles that you will ever see, just from the sleep data. The parturition codes consist of seven labor-unique behaviors. The coding system focuses on these and a few other behaviors such as grooming, activity, inactivity and sleep. Some animals sleep until 10 minutes before they deliver; all of a sudden they are in hard labor and out comes a kid. Most of them, however, go through a fairly consistent sequence, starting about an hour before delivery, what we call second stage delivery, when you can sight contractions. That is usually half an hour to an hour before delivery occurs. All this is done from video tape.
- A. I know what I would like to do with rats, but in the face of the animal welfare concerns, there are debates about commitments to large primates. I think the kind of work you do is defensible.
- S. That's what I think.
- A. NASA ought to be showcasing certain kinds of work. Could I suggest that they might want to talk to you about it?
- S. I would be interested in doing this kind of parturition work for sure. If they wanted to do some other kind of primate behavior studies, I would be interested in that, too. But, intellectually, at this point, this is what I am truly interested in, because I can think of a lot of radical reasons why it is interesting besides just the obvious practical reasons, is this whole thing of parturition.
- A. Can you list some of those theoretical reasons?
- S. Well, we should know more about these incredibly strong reflexes on the part of fetuses and newborns, the negative geotaxes, the relationship between fetal adrenal and fetal movement patterns and what may be important in initiating parturition in the first place are certainly relevant here. Also, diurnal cyclicality and the fact that almost all these births are at night, which is undoubtedly related to some kind of probably hormonal cycles, but maybe neurotransmitter cycles, for all anybody knows, since they don't know really much about it, are fascinating potential issues.

J



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HISTORY OF FEMALE MAMMALS IN SPACE

AND

NEUROEMBRYOLOGICAL STUDIES IN SPACE

An interview with Dr. Ken Souza (NASA - Ames Research Center)

Conducted by J. Alberts

(Dr. Souza was the U.S. Project Manager of several Cosmos flights, including Cosmos-1129, which included the first attempt to mate mammals in space. He is also a PI of an embryological study to be conducted on Spacelab-3)

- A. My impression is that the total census of female mammals to have been in space consists of: five Cosmos-1129 rats, two Soviet cosmonaut women, Sally Ride, and an Apollo 17 pocket mouse -- no other female mammals have been flown.
- S. That's correct, except there were also the monkeys...no, they were male, even Bonnie. I was thinking of Bonnie, but even Bonnie was male. So that's correct.
- A. When did Bonnie fly?
- S. Bonnie flew in Biosatellite 3 in 1969. Bonnie was a male. I believe the dog, Leica was also on board.
- A. Do you think that there's good scientific reason for wanting a better balance? That is, to see if there are fundamental differences in female and male physiological responses?
- S. Oh yes, there's no question. It's got to be done. We must extend the data base, especially to cover and to extend the population we are able to fly in space. There's active research, that's beginning. The female rats flown up in Cosmos-1129 were the only female mammals flown in an experimentation; that's being repeated now with the next Cosmos flight, of course. On Spacelab-4, the crew that may include a payload specialist with a female or two - that's a good possibility, and on the Spacelab-3, there should be females too. Yes, females will definitely becoming frequent space travellers. We'll need a better data base.
- A. Would you review again the problem that you're addressing in your Spacelab-3 experiment?

S. The amphibian egg is a polar egg. It has a heavy, yolk-filled hemisphere and a lighter, so-called "animal hemisphere." And at the time of fertilization, a vitelline membrane lifts off and circles the egg; granules on the surface of the egg rupture leaving a space between the membrane and the actual cortex surface with fluid. Once that happens the egg then is free to rotate in the membrane and does so, aligning itself with respect to gravity. Now, this has been observed for well over a century. Beginning at the end of the nineteenth century, people tried to keep the egg from rotating, in order to determine whether it was an essential feature of embryogenesis. People would attempt to squeeze the egg between two glass slides and hinder the rotation; sure enough, once they did that they got large numbers of abnormalities. One peculiar abnormality was twinning. People also did things like taking the egg right after fertilization and putting them in a tumbling water to disorient the developing embryos. There is an increase in abnormal development after this treatment. The question is, is the stress of disrupting the orientation really conducive to the general health of the embryo? Recently some very ingenious experiments took the jelly off the egg, embedded the egg in a liquified gelatin which solidifies, orienting the egg in whatever to a position desired, and allowed to develop. The eggs develop normally. However, one needs to remove gravity entirely to answer the essential question. As a corollary, it was noticed that at least in ontogenesis the basic organization may be affected by where the sperm enters the egg. The sperm causes a consolidation of pigment - you have a dark spot, you can even visualize where the sperm enters. Where the sperm enters determines where the egg polarizes and divides. You can determine the dorsal. If you draw a line between the sperm entering through the egg nucleus at the very northern pole...

A. Given the pressure of gravity?

S. Right...the egg nucleus lies in the northern most pole of the animal hemisphere, regardless. You can see it. If you then draw a line from sperm entry point to egg nucleus, you will then, 180 degrees opposite, identify that line of which the neural tube will form.

A. That's beautiful!

S. Now in the laboratory that formation is plus or minus about ten degrees. And one hypothesis is that it's the rotation that's produced by gravity and the position the egg is in at the time of fertilization that determines how much cytoplasmic reorganization occurs within the egg and how much variation there is in that spot or axis - whether it's plus or minus one degree, or plus or minus ten degrees... depending on what that egg is. So that's the kernel of the experiment in space.



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NEUROEMBRYOLOGICAL DEVELOPMENT IN SPACE

An interview with Dr. Ronald Oppenheim (Bowman-Grey Medical School)

Conducted by J. Alberts

- O. I recommend looking at normal parameters of development of some of the spinal nuclei; perhaps looking with pathway-tracing techniques at the development of some of the proprioceptive inputs. In the spinal cord, one question is whether things change in terms of the distributions of pathways or dendrites as a result of weightlessness.
- A. So far, what I gather is that you're looking at the space station situation, as one in which a normal sensory input would be eliminated or reduced.
- O. Yes, that's the major sort of approach that I could think of that might be worth looking at under those conditions.
- A. So am I correct in inferring that you don't predict big differences in neurogenesis, differentiation or organization of the nervous system? You don't see intrinsic organization as dependent on gravitational cues, independent of the specific forms of sensory stimulation or function?
- O. Well, again, there is pathway formation. There you may have a situation where the growing neurons or the tips of them may be subject to these kinds of things that have nothing to do with sensory input. It just may be a general gravitational field which in fact play a role in orientation. So I would say, both things: that there may be early stages when neurons are migrating and there may be alterations in their cell bodies, and a slightly later stage when they're putting out axons and trying to find their targets. Those two stages may both be of interest to look at, and wouldn't have anything to do with sensory input. Then there would be the other question of these systems which do, in fact, mediate proprioception. You can look to see whether modulations play a role in certain aspects of their conductivity.
- A. Can you imagine problems with the ventricles and cerebrospinal fluid resulting from null gravity that would alter its function or distribution?

I was thinking about it because neuronal proliferation takes place so close to the ventricle, anything that would happen right at that interface...

- K
0. My understanding is that even in severe conditions like hydrocephalus, the defect doesn't really result in any changes in the number of nerve cells; that pretty much goes on normally....

There are specific systems which one could choose, if anything's going to change, they might and it would be fairly easy to look at neuro-anatomically and quantify...and would give you a fairly quick answer to whether that's something you might want to pursue.

- A. What research questions or programs might NASA want to establish in anticipation on doing any of these various kinds of standard experiments that you're talking about? Is there parametric work that has not been done?
0. Well, I'd have to go back and look over some of the literature, but I think, for example, that the spinal system has already been pretty worked out in terms of its development in the rat...

I think one important level would be to be just purely descriptive, using selective staining techniques just to describe light microscope and electron microscope levels various kinds of muscle spindles for example. That in itself could provide a basis for comparing what happens in space. Then beyond that, using more sophisticated anatomical techniques to identify.