

Development as Adaptation: A Paradigm for Gravitational and Space Biology

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Abstract

Adaptation is a central precept of biology; it provides a framework for identifying functional significance. We equate mammalian development with adaptation, by viewing the developmental sequence as a series of adaptations to a stereotyped sequence of habitats. In this way development is adaptation. The Norway rat is used as a mammalian model, and the sequence of habitats that is used to define its adaptive-developmental sequence is (a) the uterus, (b) the mother's body, (c) the huddle, and (d) the coterie of pups as they gain independence. Then, within this framework and in relation to each of the habitats, we consider problems of organismal responses to altered gravitational forces (micro-*g* to hyper-*g*), especially those encountered during space flight and centrifugation. This approach enables a clearer identification of simple "effects" and active "responses" with respect to gravity. It focuses our attention on functional systems and brings to the fore the manner in which experience shapes somatic adaptation.

We argue that this basic developmental approach is not only central to basic issues in gravitational biology, but that it provides a natural tool for understanding the underlying processes that are vital to astronaut health and well-being during long duration flights that will involve adaptation to space flight conditions and eventual re-adaptation to Earth's gravity.

Introduction

All multicellular organisms develop, that is they undergo growth and differentiation. But growth and differentiation are abstractions. What grows when and, more importantly, what kinds of functions emerge is the actual development. To get from abstraction to the direct analysis of an organic

process requires a framework. In the present chapter, we offer one such framework. Put simply: *Development is adaptation*.

Here is what we shall do: First we review briefly the concept of adaptation, recognizing that it has several distinct, but legitimate meanings, more than one of which is pertinent to our topic. This is an important preliminary step, because it is critically important that the uses of the term adaptation be recognized at each stage of the discussion.

Next, we present an overview of how development is adaptation. Here we focus on a single mammalian species, the Norway rat (*Rattus norvegicus*), though the perspective that we offer is applicable to other species. Once this perspective is defined in a preliminary way, we go into each of these ontogenetic habitats and discuss some examples of how the developing rat adapts to it. We then turn to related issues in gravitational biology, drawing on empirical data from space flight and centrifuge studies, as possible. For each of the developmental habitats we attempt to integrate gravity-related data and issues with the larger, adaptive-developmental framework.

Finally, we discuss how such developmental analyses are pertinent to gravitational and space biology in general, and to contemporary problems in space biomedicine in particular.

Meanings of adaptation

Adaptation is a central precept of biology. Etymologically, the word derives from *ad* (toward) + *aptus* (fit). It implies a condition in which there is a match or a fit of one feature with another. Listed below are some examples of different, legitimate uses of the terms “adapt” and “adaptation,” based on a particularly lucid discussion by Pittendrigh (1958). Note that these are distinct, but inter-related uses:

- (1) *The relation between the organism and its environment.* Here we say, for example, that rats are adapted to living in burrows. This usage implies a variety of specializations that enable rats to be fit for a particular habitat. Such relations are asymmetrical in the sense that organisms are adapted to environments and not vice versa.
- (2) *A feature of the organism that serves some proximal end.* The organismal “features” denoted here can be anatomical, physiological, or behavioral and the “proximal end” or the function served might be the acquisition of food or a mate, hiding cryptically, attracting attention, or some other function that can be discerned. Thus, we might note that the intestines of infant mammals are histologically distinct. The cells and the enzymes in the intestinal lining are specially adapted for utilizing a high fat, liquid diet such as mother’s milk.

- (3) *The process of acquiring adaptation within the lifespan of an individual.* Rats that are exposed to cold conditions during development usually display greater capabilities to generate metabolic heat as adults. The development of insulative fur can be similarly influenced. Such adaptation is sometimes called “somatic adaptation” which is meant to indicate that there is somatic change without change in the genotype, whereas meanings 1 and 2, earlier, imply an historical change in genotype. The capability to adapt this way may itself be considered an adaptation, in the sense of meaning 2.
- (4) *A historical process whereby adaptation is shaped, usually by natural selection.* If we were to note that some rodents adapted to life in arid desert environments we are recognizing processes that spanned many generations and there is implied a change in genotype.

These different uses are common and commonly confused. We will see later in this chapter, and in other chapters in the present volume, that somatic adaptation (meaning #3) is a common usage in the language of space biology where much attention is given to somatic changes that occur when terrestrially born (not to mention terrestrially evolved) organisms are placed in a weightless or fractional-*g* environment.

To make matters slightly more complicated, we must now consider how the term adaptation appears in developmental thinking.

Development as adaptation: A mammalian model

Mammals, as a class of vertebrate species, are defined largely in terms of their reproductive and developmental processes. Fertilization of eggs by sperm is internal. Gestation of the offspring occurs within the body of the female. The young are born live and free (as opposed to other species in which offspring are born still encased in a developmental envelope such as an egg or shell). Mammals are uniquely and universally equipped with mammary glands by which the post parturient females provide nutrition to their offspring with mother’s milk. The young, in turn, are uniquely equipped to suckle. Provision of mother’s milk is typically part of postnatal parental behavior, i.e., coordinated activities that increase the probability of the offspring survival after it leaves the female’s body (Lott and Dale, 1973).

Features common to one group and absent in others are excellent exemplars of shared ancestry. This, coupled with specialized variants of shared general features, emphasize underlying historical processes, often but necessarily implying some changes in genotype, that lead to systematic changes in phylogeny. Developmental changes are usually the avenue of the alterations in phylogenetic patterns (e.g., Gould, 1977; Raff, 1996).

The broad and general considerations reviewed earlier, translate here into a general framework and a specific model. The general framework is that the mammalian reproductive and developmental processes create a distinct and invariant sequence of environments to which the developing mammal adapts. These environments are, in effect, definable and demanding habitats through which the young mammal moves in time. To meet the demands of each habitat requires suites of morphological, physiological and behavioral adaptations in the fetus, newborn, infant, and juvenile. As the propagule moves from one habitat to the next, it must change. In some cases these changes are gradual. In other instances they are dramatically transformative. Some commentators have even likened some of these transitions to metamorphosis, to emphasize their rapidity and drama (e.g., Yeh and Moog, 1974).

In this general framework, development can be characterized as a sequence of adaptive changes to a sequence of habitats. Unfortunately, further discussion of these ideas is beyond the bounds of the present chapter, but the interested reader may relate this framework to kindred constructs such as “ontogenetic adaptation” (Galef, 1981; Oppenheim, 1981; Alberts and Gubernick, 1985; Alberts and Cramer, 1988) and “ontogenetic niche” (Alberts and Cramer, 1988; West *et al.*, 1988).

A model and a model organism

The specific model to which we now turn will extend our discussion of the basic framework and enable consideration of data and topics in space and gravitational biology. Specifically, we shall use the domesticated Norway rat (*Rattus norvegicus*) as a “model organism.” Conveniently, the Norway rat is a standard subject for many basic and biomedical studies and it has been used extensively in space flights and ground-based gravitational studies.

Figure 1 illustrates the basic model used here. It depicts four representative scenes in the ontogenetic process of *R. norvegicus*. Importantly, it indicates these scenes as a sequence. More specifically, each of the images in Fig. 1 (A–D) represent a habitat in which a developing rat lives or, in an active and adaptive sense, “makes its living.” In this way, the rat has a job to do and it accomplishes its job with adaptations as a tool. In the process, the developing rat adapts to each habitat.

Umwelt and its roles in ontogeny

We are about to see that, in addition to the fixed sequence of habitats that mammals confront, there is also a fixed sequence to the onset of sensory function. In Norway rats, the onset of function of some sensory systems occurs prenatally, whereas other modalities begin to function at different points postnatally (Alberts, 1984). Thus, some systems are functional in the Uterine Habitat (Fig. 1A) while others are completely non-functional there. As the

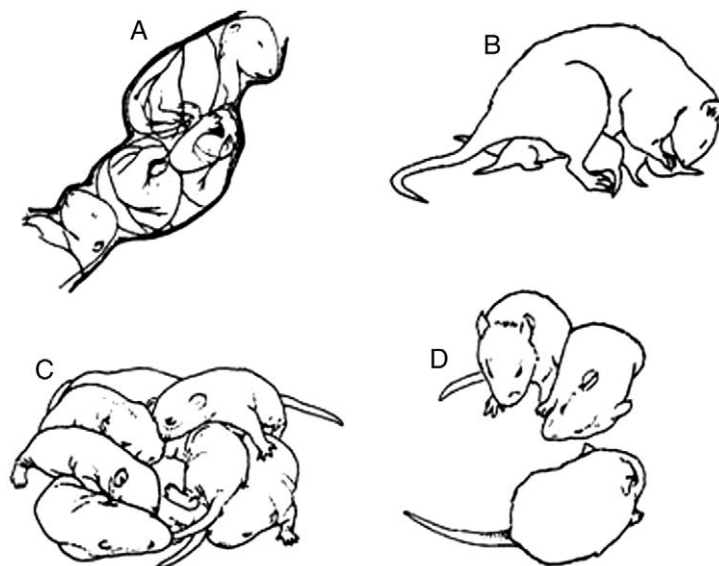


Fig. 1. Four habitats that characterize early adaptation in Norway rats. (A) The uterus as habitat. (B) The mother as habitat. (C) The huddle as habitat. (D) The coterie or social cohort.

pup enters subsequent habitats, additional sensory channels open to stimulation. In every case there is further development. These are critical points, especially within the kind of framework that we are using here.

There are two important dimensions to each habitat. One is the physical dimension, which can be described in terms of its measurable parameters. The other important dimension is the organism's *experience* of the habitat, as determined by the organism's sensory-perceptual systems, i.e., the functional effects of the stimuli that reach the organism via its sensoria.

In the scientific study of animal behavior, or ethology, the concept of *Umwelt* (Uexkull, 1909), which denotes the perceptual world of an animal, is an important topic. The *Umwelt* of different species varies dramatically, depending on the kinds of sense organs that they have. (Some bats discriminate objects by their echoes, other animals use magnetic fields to orient or navigate, while others "see" heat with nasal pit infrared detectors. Many animals perceive olfactory cues with greater acuity and in dimensions not available to humans.)

Umwelt is applicable to our developmental concerns here. The sensory capabilities of fetuses and other immature forms are often dramatically different (usually more limited) than in the adult form. Such differences are important in understanding behavior at each stage of development. In addition, contemporary behavioral neuroscience has established that experience [sensory function = experience (cf., Gottlieb, 1971)] plays important, determinative roles in subsequent neural and behavioral development. The celebrated studies of Hubel and Weisel, (1998) on visual system development emphasized the

critical roles of visual stimulation in the development of the visual system, including formation of cell assemblies and synaptic architecture in central nuclei, as well as the development of visually guided behavior. The rule is that function and development are bidirectionally linked (function $\leftarrow \rightarrow$ development). More recently, there is accumulating new evidence of *inter-sensory* development, i.e., that stimulation and function in one modality affects the development of other sensory modalities (e.g., [Kenny and Turkewitz, 1986](#); [Lickliter, 1993](#); [Reynolds and Lickliter, 2004a,b](#)).

There is an invariant sequence of onset of function for the sensory systems that have been analyzed in comparative developmental studies ([Alberts, 1984](#); [Gottlieb, 1971](#)). The sequence is: Tactile–Vestibular–Auditory–Visual. The chemical senses appear to be among the early onset systems [in rat, probably after vestibular onset and well before auditory and visual (cf., [Alberts, 1984](#))]. Specifically, on the basis of anatomical, physiological, and behavioral evidence it appears that by embryonic day 16, the fetal rat reacts to punctate tactile stimulation to selected regions of the body ([Narayanan et al., 1971](#)). Vestibular function is also apparent prenatally, as are the chemical senses (see [Alberts, 1984](#)). But, in every case, this is simply onset of function. The subsequent timing, rate, and extent of further development (e.g., threshold sensitivities, range of detection, topographic spread, etc.) appears determined, at least in part, by experience. Auditory and visual function in rat have postnatal onsets, around postnatal day 12 and 15, respectively.

To summarize briefly: in *R. norvegicus*, tactile, vestibular, and chemical sensing begins prenatally and continues after birth. Thus, we can ask whether and to what extent the fetus experiences the tactile, vestibular, and chemical features of the uterine habitat. Similarly, we must extend our questions into a rat's postnatal life. Auditory onset of function begins around postnatal day 12 (P 12). As the pups approach the time of egression from the nest, the eyelids unseal and onset of vision is initiated (about P 15). Although all sensory systems are operational by P 15, development continues thereafter.

For our present concern with gravitational questions, the early onset of vestibular function is profoundly important. Several implications should be noted. First, we emphasize that the onset of vestibular function is dramatically early in every vertebrate species that has been studied. Thus, all sensory systems that develop subsequently are, in theory, susceptible to influence by the previously initiated vestibular function. Perhaps this makes it less surprising that the vestibular system is integral to visual function, to proprioception, to spatial learning, and in other non-vestibular systems. But mysteries abound. Precious little is known about the development of vestibular function itself, and the prospect of finding that the development of other, perhaps *all* other sensory–perceptual systems may be influenced by vestibular development is truly exciting, if not sobering.

With these considerations in mind, we can now enter the rat world(s) and consider development as adaptation.

The uterus as habitat and the fetus adaptations

Figure 1A depicts the initial habitat in which all prenatal mammals reside. It is the mother's uterus and this figure shows a few fetal rats *in situ*. Norway rats, like most mammals, produces multiple offspring with each parity. In rat, there are usually about 10 fetuses, arranged in line in each of the two uterine horns. The figure shows just one portion of one horn. Each fetus resides individually in an amniotic sac and, in the model used here, this is its prenatal habitat.

Consider what a special and demanding habitat this is. Essentially, it is an aqueous habitat for the fetus (though, as a presumptive rat, this is an organism destined for terrestrial life in gaseous habitat). In the uterus, the fetus is physically connected to the uterine wall, tethered by the umbilical cord and placenta. The cord is a conduit for arterial and venous circulation and supports homeostatic functions including respiration, nutrition and waste removal. The uterine habitat changes considerably during the three weeks or so gestation, but again, this is beyond the scope of the present presentation and the interested reader can consult many sources for full discussions (e.g., Dawes, 1968; Liggins, 1982).

It is in the uterine habitat that the rat begins its behavioral life, that is, movement begins. These events are lawful, organized, rhythmic, and functional. Breathing movements begin prenatally, though it is lung fluid rather than air, that is moved through the trachea (Dawes, 1974; Oliver, 1981). Although unrelated to prenatal gas exchange functions, fetal respiratory movements contribute to the remarkable postnatal ability of the respiratory musculature to maintain vigorous, continuous, coordinated function after birth (Liggins, 1982; Dawes, 1968).

Trunk and limb movements also begin prenatally. Reductions in embryonic limb movements, even for a relatively brief period, can permanently block joint flexibility. Limb movements are part of the formative process of the musculoskeletal apparatus (Drachman and Sokolov, 1966; Moessinger, 1983). Similarly, manipulations that disrupt fetal swallowing and tongue movements have been directly associated with impairments in gastrointestinal development (Liggins, 1982).

The fetus has two jobs: It must “earn a living” (i.e., adapt) in an aqueous, restrictive, and uniquely structured habitat—a uterus, while busily becoming an entirely different type of organism, an orally feeding, air-breathing quadruped. The phenomena of prenatal movements provide multiple lessons. First, we can ask whether the movements are an aspect of adaptation to the uterus. Fetal swallowing contributes to the reduction in amniotic fluid volume, a mechanical necessity as the propagules grow within the confines of the dam's body. Movements appear to be part of successful (and adaptive) growth and differentiation in the uterine habitat. Interrupting the expression of such movements can compromise the formation of functional joints or disrupt the

development of other systems. Thus, movements in utero contribute both to immediate and future development.

The uterus is a stimulating environment. The uterine habitat is contained in another body, that of the mother. Many fancy that such an encased habitat must be the epitome of quiet, serene, undisturbed existence. Not so.

There is now a sizeable body of evidence showing that uterus is bombarded by stimuli. Maternal behavior and maternal physiology are major contributors of the stimuli (e.g., Bradley and Mistretta, 1975; Decasper and Fifer, 1980; Hofer, 1981; Fifer and Moon, 1988; Ronca *et al.*, 1993). For example, we (Ronca *et al.*, 1993) have shown that during gestation, fetuses *in utero* are exposed to tactile and vestibular stimuli associated with the mother's behavior. Specifically, fetuses are exposed to accelerations as the dam ambulates, circles, and rears. When she grooms her abdomen or lies prone, the fetuses are compressed, and they are vibrated as she scratches with a hindlimb. These activities occur at high levels throughout pregnancy, exposing fetuses to *hundreds* of sensory inputs each day, many of which occur within just 24 h of birth (Ronca *et al.*, 1993).

During labor, fetuses are pitched and rotated by uterine contractions. They are exposed repeatedly to powerful compressions of the head and body until the newborn is finally squeezed through the birth canal. Upon delivery from the birth canal, the dam removes the birth membranes, enabling air to reach pups' nares for the first time. She licks and handles pups, removing amniotic fluid from the skin as she lifts and rotates them, providing extensive cutaneous stimulation and repeated angular accelerations. The postpartum thermal environment is much cooler than the intrauterine environment (21°C vs. 37.5°C), causing thermally fragile newborn pups to cool to room temperature within minutes of birth. They are soon re-warmed, however, when they are gathered in a nest and brooded over by the dam.

In an earlier section of the present chapter, we discussed the onset of sensory function in vertebrates, noting that in rat, tactile and vestibular sensitivity have prenatal onsets. A pertinent question, then, is whether the fetal rats' initial sensory capabilities are sufficient to detect the stimuli that impinge upon them *in utero*? We asked this question by first measuring the stimuli that reach a rat fetus *in utero* (Ronca *et al.*, 1993). We then created simulations of these stimuli and presented them at realistic levels to fetal rats that had been externalized from the mother's body, while maintaining the umbilical connections. We measured stimulus detection by the fetuses via changes in heart rate and behavior that had been documented as valid and reliable measures (Ronca and Alberts, 1990). From the tests with simulations, we learned that fetuses do indeed experience the vestibular, tactile, chemosensory and thermal stimuli associated with life in the uterus and parturition (see Alberts and Ronca, 1996; Ronca and Alberts, 1994). Such experiences are involved in fetal learning, arousal, and birth-associated reorganizations that are vital to perinatal adaptation.

Demonstrations of responses to vestibular stimuli in fetal rats are exciting data for space biology, for it is currently more feasible to fly pregnant rats than to sustain a lactating rat and her suckling offspring in the weightless environment of orbital space flight. With this in mind, we turn to reflect on past flight experiments, which have provided some enticing findings.

Gravid without gravity: Consequences of space flight during gestation

To date, there have been three occasions in which prenatal development of rats has been challenged with space flight conditions. The first such flight experiment was on Cosmos 1514, launched in December 1983. This was followed by two Small Payload experiments flown on the Space Shuttle (STS-66 and -70) in 1993 and 1995, respectively. Figure 2 illustrates some of the mission parameters (for additional detail, see Ronca, 2003). Postflight studies included analyses of the mothers as well as the offspring. In each of these investigations, some of the fetuses were studied soon after they returned to Earth and others were left to gestate and then were studied as newborns and postnatally. In the present section we shall discuss the studies of the fetal development and the gestating dams.

Fetuses from Cosmos 1514

A major goal of the Cosmos 1514 mission was to test the ability for mammalian pregnancy and prenatal development to proceed in microgravity (Alberts *et al.*, 1985, Serova, 1993). The unmanned satellite carried ten, time-mated pregnant rats into low Earth orbit. The fetuses inside these Flight rats were the first mammalian specimens to undergo a portion of their gestation in the absence of Earth-normal gravity. All of the ten pregnant Flight dams returned alive and still pregnant.

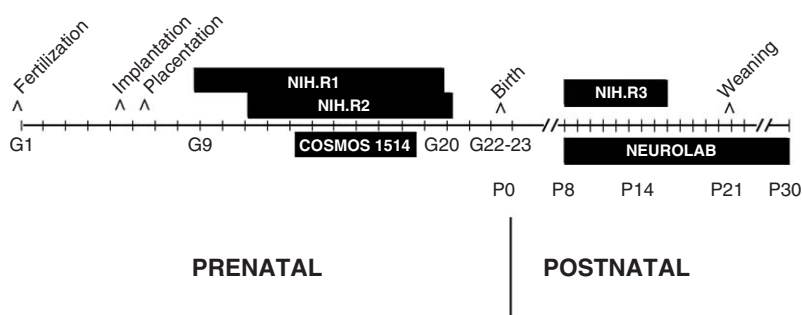


Fig. 2. Overview of spaceflight missions that carried prenatal and young postnatal rats in relation to developmental milestones (G = gestation day; P = postnatal day).

Five of the ten Flight dams were sacrificed at Recovery and the remainder were permitted to undergo parturition, which was accomplished successfully by four of the five dams. Fetal offspring that underwent a portion of their gestation (about 20%) in space were morphologically intact and appeared on a gross level to have grown and developed properly. The Cosmos 1514 flight fetuses weighed approximately 10% less than control fetuses at recovery and flight dams weighed approximately 18% less than control dams (Serova *et al.*, 1993). Analyses of fetal tissue revealed mitotic figures in cortical locations that were interpreted as signs of retarded cellular development and migration (Keefe, 1985). The failure of pregnant Flight dams to gain weight normally compromised interpretation of any seemingly anomalous outcomes in the offspring. Nevertheless, the fact that space-flown fetuses were intact and appeared to be well developed was itself an extraordinary finding and it left open the door to additional explorations.

Pregnant dams on NIH.R1 and NIH.R2

The NIH.R1 and NIH.R2 were experiments flown in mid-deck lockers of the Space Shuttle. Rats were housed in Animal Enclosure Modules (AEMs), which are sealed so the rats are inaccessible to the crew, except for visual access through one Lexan surface. As shown in Fig. 2, these missions were 11- and 9-days-long, and were timed to impose microgravity conditions during the second and third weeks of the rats' 22-day pregnancy.

Weight gains in pregnant dams during the NIH.R1 and NIH.R2 flights were identical to or very close to that of the ground controls (Burden *et al.*, 1997; Ronca and Alberts, 2000a), which enhanced interpretation of the measures made in the offspring, compared to the earlier results from the Cosmos mission.

NIH.R1 was the first time video data were collected systematically and analyzed as part of an experiment protocol, and the effort paid great dividends for several investigators. It also provided valuable information that will contribute to future experiments as well as to hardware design. Even in these short, 7- to 15-min-long video segments, Flight dams were observed to eat, drink, groom and interact with one another. Species-typical behaviors and movements were observed in both Flight and Synchronous control dams. It was clear that under flight conditions, the dams ambulated about the full volume of the AEM, using their paws and legs on the walls and surfaces to propel themselves throughout the habitat.

Although Flight dams appeared to adjust to weightlessness, we found that their behavior during space flight differed from that of the ground controls. This was seen when we applied a self-referencing movement notation system, by which we classified each movement of the dams in relation to the posture of the body at an immediately preceding time-point (Alberts and Ronca, 1997). The results of this analysis indicated that movements involving pitch and yaw were about equivalent in Flight and Control dams. In contrast, Flight dams

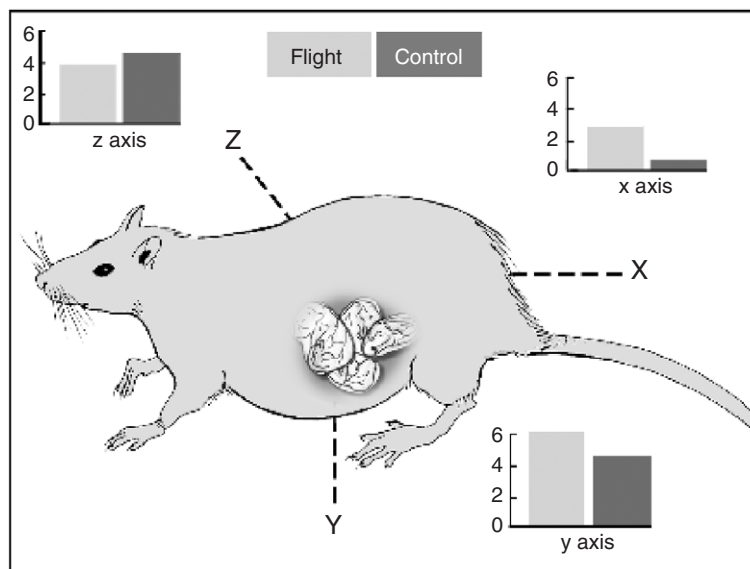


Fig. 3. Relative rates of body movements, measured in self-referenced transitions from an immediately previous timepoint, and expressed on three vectors (X, Y, Z) of movement. Data were from time-matched samples of behavior of pregnant dams housed in the AEM during orbital flight and from Synchronous Control dams housed in an environmental chamber that was yoked to temperature and humidity conditions of the Space Shuttle middeck.

displayed about seven times more rolling movements than did Controls. This difference, we think, was a consequence of the increased numbers of surfaces available in microgravity for walking and crawling. Many of the rats' movements from surface to surface involved rolling movements on the longitudinal body axis (see Fig. 3).

These flight-induced alterations in maternal movements changed the pattern of prenatal vestibular input to fetuses *in utero* and were associated with striking changes in fetal vestibular morphology and perception (Ronca *et al.*, 2000).

Fetuses from NIH.R1 and NIH.R2

Several investigators who analyzed fetal tissues from NIH.R1 and R2 discovered differences between space flight- and Earth-gestated fetuses. Among the noteworthy findings were:

- (a) R1 fetuses showed a six-fold increase in atrial natriuretic peptide (ANP), a cardiovascular-related hormone that regulates sodium and water excretion, and vasodilatation (Davet *et al.*, 1999). This observation is similar to reports of increased ANP in the astronauts, presumably related to inflight fluid shifts.

- (b) R1 fetuses also showed delayed development of the choroid plexus, responsible for cerebrospinal fluid (CSF) secretion and involved in brain homeostasis (Mani-Ponset *et al.*, 1997). Significant immunocytochemical changes were observed in the distribution of proteins involved in cell differentiation and CSF production within the choroidal epithelial cells.
- (c) R2 flight fetuses evinced changes in the developing superchiasmatic nucleus (SCN), which is the reception area for photic information that entrains circadian rhythms to the light/dark cycle. Flight fetuses SCN appeared developmentally delayed (Murakami *et al.*, 1997), a finding that fits well with the host of disturbances in the circadian timing system observed in astronauts (Dijk *et al.*, 2001).
- (d) Sonnenfeld and colleagues (Sonnenfeld *et al.*, 1998) reported no changes in immune responses of R1 flight fetuses, despite significant immune suppression in the dams that was characteristic of that seen in non-pregnant adult animals.
- (e) R2 Flight and Control fetuses did not differ in key proteinases important in bone development and remodeling (Davis *et al.*, 1998).

NIH.R2 included a study designed to assess the functional status of the fetus' vestibular system prior to re-adaptation to 1-g. This was accomplished by applying the procedures described earlier in which live fetuses are externalized from the mother's body and tested for sensory responsivity (cf. Ronca and Alberts, 1994). Flight fetuses were tested about 2 h after Recovery from orbital space flight. They showed magnified responses to vestibular stimulation (rolling through a 70° arc) compared to Control fetuses tested identically. This finding was particularly surprising because previous data (from NIH.R1) had prepared us to expect an attenuated response to vestibular stimuli, as evidenced by the water immersion righting responses of the neonates. Nevertheless, we were better able to understand the dramatic hyper-responsivity of Flight fetuses to angular accelerations after we analyzed the video recordings of the Flight dams and Control dams (Alberts and Ronca, 1997). The video data revealed differences in the mother's behavior that created contrasting adaptive challenges for the fetuses.

Postnatal pups that experienced prenatal space flight

Cosmos-1514, NIH.R1 and NIH.R2 together provide a broad, preliminary picture of how space or microgravity adaptation may proceed during early, formative phases of the body, brain, and behavior. Four dams from Cosmos-1514 delivered litters that were used for postnatal tests. The satellite flew from G13 to G17.5, so that gestation in these dams continued for about as long as the mothers and fetuses had been exposed to microgravity. For NIH.R1, newborn rats were derived from ten dams that gave birth vaginally on G22

following unilateral hysterectomy (removal of one of the dam's paired uterine horns) on G20. For NIH.R2, newborn rats were available from six of the ten intact Flight dams. They were allowed to undergo vaginal deliveries.

In all three missions, litter sizes were similar between the Flight and Control conditions. The pups' external appearance was appropriate for their calendar ages. Average birth weights of Flight pups did not differ from Synchronous controls. In the Cosmos rats, however, pup weights were strikingly variable in the Flight group. Body weights of NIH.R2 pups were modestly but significantly less than Synchronous controls until the 14th postnatal day (Hoban-Higgins *et al.*, 1999; see also Ronca, 2003). This outcome may have been related to the minor (5%) reduction on the dam's body weights of Recovery. No differences in body weight were observed thereafter (through P90) for either NIH.R1 or NIH.R2 offspring.

Postnatally, the emergence and development of locomotion and gait appeared equivalent for Flight and Control pups through P 81, when testing was terminated (Wong and DeSantis, 1997).

We (Ronca and Alberts, 2000b) analyzed vestibular-mediated responses from Day of birth (P0) through P5. The contact righting reflex, composed of stereotyped movements that rotate the body from supine to prone on a solid surface, did not differ in Flight and Control pups. This proved to be important evidence, confirming that Flight pups could perform the coordinated movements involved in righting the body to prone. Such tests of "surface righting" present to the pup abundant tactile and proprioceptive cues, in addition to the vestibular stimulus, when the pup is held on its back. We therefore included tests involving "water immersion righting." In this preparation, the tactile and proprioceptive cues are eliminated and the challenge to the pup becomes much more purely vestibular, presumably testing its perception of linear acceleration—or gravity. Here the pup is rotated to supine, held, and then released just below the surface of a warm waterbath. The pup's buoyancy allows slow descent and its "diving reflex" prevents aspiration of water. This is designed to be a test of vestibular sensitivity of gravity.

Flight newborns were deficient in responding to the vestibular perturbation on P1 and P3 whereas Control pups righted themselves quickly and reliably in the immersion tests. The same Flight pups were demonstrably capable of surface righting, so we were certain that their failures in the immersion test were not due to motor deficits. We believe the Flight pups returned from space relatively insensitive to gravitational cues. Their deficit was profound, but transient, as evidenced by complete response recovery on P5.

Collectively, the emergent picture is enticing. Removal of gravitational cues during the early phases of vestibular development can eliminate sensitivity to gravity. At the same time, sensitivity of angular accelerations may be magnified. We are aware of two, non-mutually exclusive contributions to such a phenomenon. First, the pups' habitat (the uterus) is affected profoundly by the mothers' weightlessness. The fetuses are without forces of linear

acceleration (gravity). But, because the mothers are active in space, the fetuses are nonetheless subjected to forces of angular acceleration. Moreover, and importantly, the Flight dams *moved differently* than did the Controls. Their movements included much more rolling, in addition to pitch and saw movements. Thus the fetuses' space must adapt to deprivation of gravity cues and an *enrichment* of inertial cues. We believe we observed evidence of precisely this unique form of adaptation.

The second kind of contribution to the pups' behavioral adaptation was presented on the level of neural growth.

Gravid on the gravity continuum

Gravity is a graded stimulus. Yet, for many years, space flight opportunities have strongly influenced the kinds of data that have been available and thus have led many to focus on comparisons of Earth-normal gravity (1-*g*) and its absence, as approximated by orbital space flight (0-*g* or micro-*g*). Rigorous understanding of gravity as a biological factor will be attained, however, only after we can describe and explain the form of biological processes, as they are over a range of gravitational forces, ranging from 0-*g* through fractional *g* levels below 1.0 and above it, say to 2-*g*, which happens to encompass the range most pertinent to human and animal space travel.

In recent years, ground-based studies of early mammalian development have been conducted using centrifugation at varying *g*-loads in excess of 1-*g*. Two common functions can describe many (but not all) of the observed responses to deviations from 1-*g*: some physiological and behavioral systems respond to hypo- and hypergravity in opposite directions, thus providing a somewhat continuous function from 0-*g* to higher (>1-*g*) levels (Phillips, 2002). A classic example comes from data on antigravity muscles, such as the soleus, that shows a linear increase in volume with increasing *g*-load (Vasques *et al.*, 1998.). In contrast, there are other cases in which physiological or behavioral systems respond to deviations from 1-*g* with similar responses, regardless of whether the deviation is above or below 1-*g*. For example, when infant rats are placed in the supine position near the top of a heated water-bath and then released, they tend to rotate their bodies to a prone position. Neonatal rats that underwent gestation during space flight were impaired in their ability to perform this vestibular-based response postflight, but the response recovered several days later (Ronca and Alberts, 2000b). We recently repeated this experiment at 2-*g* and observed a similar pattern of compromised responses (Ronca *et al.*, unpublished observations).

Analyses of differing biological responses across a range of gravity vectors will ultimately lead to the establishment of general principles and the development of regression equations that will help us further delineate relationships between effects of hypo- and hypergravity. In this way, we can begin to make

initial, limited predictions for specific responses in microgravity from those studied in hypergravity.

Gravid at greater gravities

Oyama and colleagues (Oyama and Platt, 1967; Oyama *et al.*, 1985) conducted some of the initial studies of hypergravity-rearing in rodents. In those studies, young female rats and mice were adapted to either 2.16-*g* or 3.14-*g* centrifugation, and then mated. Rats that were impregnated and gave birth during centrifugation were reported to be “less maternal” and neonatal survival was greatly diminished relative to 1-*g* controls. The period around the time of birth was reported to be highly vulnerable to hypergravity exposure, with extensive neonatal losses occurring during this time. This led Oyama’s group to interrupt centrifugation for approximately sixteen hours each day beginning at birth and throughout the first few postnatal days. Offspring survival rates declined precipitously as *g*-load increased (Oyama and Platt, 1967; Oyama *et al.*, 1985; Baer *et al.*, 2000). It is interesting to note that mice born and reared during centrifugation were somewhat less affected by hypergravity exposure than were rats.

Exposure during pregnancy and birth to even modest increases in *g*-load (1.5-*g*) exerts immediate effects on dams, and is likely to affect the growth and development pups *in utero*. Initially, body mass declines, stabilizing at about 8–15% less than 1-*g* controls (Ronca *et al.*, 2000, 2001). Food and water intake (adjusted per 100 g dam body mass) were reduced in hypergravity-exposed dams relative to controls. For the first four days of centrifugation, hypergravity-exposed pregnant dams were approximately 25% less active than controls. Within just a few days, dams begin to show signs of adaptation to centrifugation. After nine days of centrifugation, late pregnant (G20/21 of the rats’ 22-day pregnancy) dams in the 1-*g* condition began to reduce their previous levels of activity, but dams in the 1.5-*g* condition did not (Ronca *et al.*, 2000). The augmented activity of hypergravity-exposed dams during late pregnancy led us to examine specific behaviors of the dams during this period. Time spent feeding, drinking, and self-grooming was comparable in the 1.5-*g* and 1-*g* dams, regardless of circadian cycle. In contrast, the late pregnant hypergravity-exposed dams spent three times more time engaged in nest-building behavior. This latter observation suggested to us that changes in patterns of maternal care might play an important role in neonatal losses during exposure to hypergravity.

We tested the hypothesis that maternal reproductive experience determines neonatal outcome following gestation and birth under hypergravity conditions (Ronca *et al.*, 2001). Primigravid (first pregnancy) and bigravid (second pregnancy) female rats were exposed to 1.5-*g* centrifugation from G11 throughout birth and the first postnatal week. On the day of birth, litter sizes were identical across gravity and parity conditions although significantly

fewer live neonates were observed among hypergravity-reared litters born to primigravid dams as compared to bigravid dams (82% and 94%, respectively; 1.0-*g* controls, 99%).

Within the hypergravity groups, neonatal mortality was comparable across parity conditions from the first to the seventh postnatal day at which time litter sizes stabilized. These results indicate that prior pregnancy and birth can reduce neonatal losses in hypergravity during the first 24 h after birth, but not on subsequent days.

In seeking to explain the hypergravity-related neonatal losses, we analyzed the dams' postpartum maternal behavior. Similar to the results of the space flight studies, there were no observable changes in the mothers' behavior during birth. The behavior of primigravid hypergravity mothers differed from the other conditions in that these dams tended to disrupt nursing bouts and pups within the huddle by frequently digging within the nest and rearranging the pups. This pattern was highly correlated with neonatal mortality ($R^2 = 0.99$, $p < 0.02$).

Our data suggest that during the period around the time of birth, the maternal-offspring system is particularly sensitive to relatively modest (0.5-*g*) increments in the Earth's gravitational field. Maternal reproductive experience appears to be a major determinant of postpartum survival in hypergravity. The newborn rat is profoundly vulnerable to changes in the Earth's gravitational field, in part through changes in the mother's behavior.

The mother as habitat

When the infant mammal emerges from the birth canal it suddenly and irreversibly enters a dramatically different world. Before birth the fetus is influenced indirectly by the mother's behavior. After birth, however, the mother's actions are more specifically directed toward the young and she becomes a central environmental factor, essentially defining the newborn rat's postnatal habitat. As each pup is born, the mother licks it, both cleaning the pup and providing it with tactile stimulation that facilitates its newborn physiology and behavior, including pulmonary respiration (Ronca and Alberts, 1995a,b), thermogenesis (Alberts *et al.*, 1992), and suckling (Ronca *et al.*, 1996; Abel *et al.*, 1998). Without such early postnatal tactile stimulation, survival is compromised.

The mother's body and the nest that she builds, become the buffer between the vulnerable newborn and the outside world. No longer embedded within her body, the altricial infant rat depends on the conductive heat and insulation provided by contact with the dam. During the first few postnatal days, the rat dam spends 60–80% of the time in the nest and in direct physical contact with the pups. Most of this time is spent nursing. Within minutes of birth, the rat fetus metamorphoses into a veritable suckling machine. The dam provides milk during nursing bouts that are punctuated by “letdowns” of milk that occur

simultaneously to all 12 nipples, even if there are fewer than 12 suckling pups. These milk letdowns are stimulated by the contraction-inducing effects of oxytocin, which is released by the maternal pituitary prior to each milk ejection (Lincoln *et al.*, 1973). The letdowns occur every 3–5 min and there may be 8–10 letdowns per nursing bout. Mother's milk is the rat pup's sole source of food and fluid for the first 17 days and pups continue to attach to nipples and suckle until day 28 or later (Thiels *et al.*, 1990).

Pups are equipped with a host of specializations for acquiring and utilizing milk as a source of nutrition, water, electrolytes, and immune competence. The shape of the pups' face and the neuromuscular patterns of facial muscle are organized around suckling (Westneat and Hall, 1992). With the mother as habitat and her milk representing the pups' sole substrate, the pup's gastrointestinal system is adapted to utilization of mother's milk. This is particularly evident in the specialized cells of the small intestine which are shaped to hold fat globules, typical of the milk diet, and also of the preponderance of lactase, the enzyme used to digest the major carbohydrate in milk. As can be seen in Fig. 6, lactase levels essentially disappear as weaning progresses and milk is replaced by solid food.

Rat pups can arouse a rat dam with their ultrasonic vocalizations (Farrell and Alberts, 2002a,b). When a dam approaches her litter of pups, it is the tactile stimulation that they deliver to her ventrum that provokes from her the arched "kyphosis" posture above the litter (Stern, 1996), depicted in Fig. 1B. Beginning *in utero*, rat pups learn the identity of chemical cues (Pedersen and Blass, 1981) which, upon birth, arouse them into nipple-searching activities (Teicher and Blass, 1977). The aroused pup moves its head from side-to-side, scanning along the dam's ventrum until it encounters a nipple. Nipple apprehension involves coordinated head and mouth movements and seems to rely on stereotyped "rooting reflexes." Once attached, the pup's mouth forms a tight, salivary seal at the base of the nipple. Suckling begins. Tactile stimulation to the dam's nipples exerts direct excitatory effects on hypothalamic neurons which summate and trigger the pituitary release of oxytocin and the subsequent milk ejection. Pups can detect the intramammary pressure associated with milk letdown and they display a vigorous extensor reflex in response to the letdown cues.

Before and between nursing bouts, the dam licks each pup, focusing her oral activity at the anogenital (AG) region. The licking behavior is highly stereotyped. Typically, the dam grasps a pup with her forepaws and manipulates it into position, which is ventrum up with the infant's head below her chin and its caudal region in front of her mouth and snout, thus exposing the anogenital region. Pups placed in this supine position would otherwise exhibit a vigorous "righting reflex" and rotate themselves to prone (Pellis *et al.*, 1996), but the tactile stimulation to the pup's ventrum inhibits this reflex and the pup remains supine and still, but for a leg-extension response that appears to augment the dam's access to the AG region (Moore, 1992).

The mother's licking triggers the pups' micturation reflex, whereupon it urinates and/or defecates. The infant cannot void spontaneously. Maternal licking clearly provides the infant rat with needed stimulation, helps with hygiene, and serves the infants in other ways (Gubernick and Alberts, 1983). But the dam does not only stimulate the needed release of pup urine; she consumes the urine. In fact, by licking the pups and consuming their urine, the dam recycles about 2/3 of the water in the previous day's milk. This coordinated exchange between the mother and the pup is vital to the dam's water and electrolyte balance (Friedman *et al.*, 1981).

If this weren't enough, there is a sex difference in how much pups are licked. Males receive about three times more licking of their anogenital regions than do their female littermates. Equivalent amounts of licking are received on the rest of the body (Moore, 1992). Male rats provide an androgen-dependent chemical cue from the preputial gland that augments the dam's AG licking. Interestingly, the effect of the AG licking is to masculinize the recipient. Thus, the male rat hormones not only masculinize its brain and body, but the same hormones affect the mother's behavior in a way that delivers additional, masculinizing stimulation to them.

We relate these research stories concerning maternal licking because they emphasize nicely the important point that the pups' adaptations to the mother (and vice versa) create a true, integrated system of dam-and-offspring that is functionally intertwined. When these individuals or the entire system is challenged to function in a novel environment, it is vital to appreciate the multiple levels of integration if one is to interpret accurately the changes that may be observed.

Maternal behavior and offspring adaptation: effects and responses along the gravity continuum

When a broad, contextual cue such as gravity is manipulated, it is often necessary to parse the consequences into two broad kinds: we must distinguish *effects* from *responses*, especially when interpretations of adaptation may be affected. To borrow from an example used by G.C. Williams (1966), a biologist whose writing has been incisive and important in evolutionary theory, consider a flying fish leaping out of the water and splashing back in. If the fish does not return to the water, he noted, it won't survive. Williams argues emphatically, however, that it is wrong to invoke biological adaptation as an explanation to account for the fish's return to the sea. In this case, returning to the water can be explained as a mere "effect" of gravity. No specialization is needed to account for the phenomenon. In contrast, an adaptive "response" is an active, organized biological process, something that has probably been shaped by natural selection. A kidney that enables a fish to return to salt water after living in fresh water is a candidate for an adaptation.

Now, the kinds of phenomena faced by contemporary gravitational biologists who might witness the very first occasion *ever* that a particular species lives in microgravity clearly cannot be considered as direct results of natural selection. Nevertheless, our task is to parse whether a phenomenon that we observe is a mere effect of weightlessness versus an alteration that involves a system that has been shaped or evolved by natural selection in a way that has incorporated gravitational forces. How that system reacts and possibly adjusts to altered gravity during the animal's lifetime (i.e., without invoking a change in genotype) may be its somatic adaptation.

We believe that developmental biologists investigating gravity-related phenomena that involve maternal-offspring systems will face the basic challenge of recognizing active responses and distinguishing them from mere "effects" of altered gravity. This was heralded by some of the pioneering efforts on the Neurolab mission. For the first time, lactating rats and their suckling litters were placed in the microgravity conditions of orbital flight. Many pups did not survive, especially in the groups launched at the youngest ages (Maese and Ostrach, 2002). Does this automatically mean that gravity is essential for the developing system to survive? No, it does not. A more direct and parsimonious explanation (the preferred type in an empirically based understanding) is that some of the effects of weightlessness in the cages used for the flight made it difficult for the mothers and pups to remain together. Absent were some of the "passive" effects of gravity that normally afford specific adaptations such as the lactational system, to transfer milk to pups, who, in turn, are applying adaptive behaviors such as nipple search and sucking responses to ingest and utilize milk. Hardware design is thus paramount in such studies, and every flight (including the so-called failures) will be a source of valuable information with which our science can be honed.

There have been too few opportunities to study mothers and offspring during the important early postnatal period. Consequently, there is little to report. The prospects are bright, however, for the earliest efforts suggest that our mammalian model is so beautifully and robustly organized, that it is capable of adjusting adaptively to microgravity and solving the kinds of passive problems that may arise. We know, for example, that weightless, suckling rat pups, given sufficient support, can attach to nipples and suckle milk. Initially, this was determined by one of us (JRA) in studies conducted on NASA's KC-135 aircraft, used for creating brief periods (approximately 25 s) of weightlessness during parabolic maneuvers. During a sequence of such microgravity episodes, milk-deprived rat pups, aged 5-, 10-, or 15-days were held near the exposed ventrum of their anesthetized mother and allowed to attach to a nipple. Then, by appropriately timing an intra-peritoneal injection of oxytocin, it was possible to induce a letdown of milk. Weightless pups exhibited the typical stretch-reflex to milk letdown (Lincoln *et al.*, 1973) and maintained their oral grasp of the nipple! This observation was extremely informative and encouraging, because

it demonstrated that a weightless pup *can* perform its role in the mother–pup suckling sequence.

Video analysis of brief inflight footage of lactating dams and litters taken during the NIH.R3 flight provide evidence that, even in weightlessness, maternal nursing and maternal care (pup retrieving and licking) are retained in space (Daly and Ronca, 2002). This was true despite the observation that, the overall coherence and dynamics of the nest were drastically changed.

Our task, as investigators, will be to create nests that will properly afford the mother the opportunity to care for the young under various gravitational challenges.

Neurolab experiences and observations are consistent with the view that the *R. norvegicus*' mother–litter system is highly robust and capable. Many of the views of the mothers and pups during that flight revealed unconstrained pups and chaotic arrangements of bodies. Nevertheless, the survival of most of the pups under what turned out to be suboptimal conditions is testimony to the species' ability to cope with novel challenges. With additional attention and experience in hardware design, it is likely that appropriately supportive environments can be designed that will enable us to study gravitational responses of the mother–offspring system.

Though speculative, we can raise a couple of examples of promising areas involving the postnatal pup living in the maternal habitat that might reveal adaptive effects related to gravity. An appropriate nest environment for microgravity studies involving nursing mothers and their developing offspring is one that will assist (in the absence of Earth-normal gravity) the coherence of the litter group, for the dam normally nurses them as a coherent unit. Within this nest, pups should be able to respond to the episodic appearances of the dam and her maternal behavior, including presentation of her ventrum for nursing. Figure 4 illustrates the head movements made by pups during their “nipple search” behaviors. In Earth-gravity, this behavior is the source of both linear and angular forces to the developing vestibular apparatus. It is probably important that such stimuli will be linked, proprioceptively, to efferent activity of the infant's neck, shoulder and forelimb musculature. These scanning movements bring the pup's sensitive perioral skin in contact with the nipple protuberance, and stimulate the grasp reflex. There follows vigorous suckling and treading, both of which contribute to the release of oxytocin from the dam's pituitary and milk letdown. It will be instructive to observe and measure such movements under various gravitational loads. We will learn about the control and regulation of such adaptive behaviors and, importantly, we will better understand the quantity and qualities of the stimulation sustained by the developing vestibular system. Nipple-shifting, mentioned earlier, will also be important to understand under novel gravitational conditions, for this may help in studies such as hippocampal development and spatial learning (Cramer, 1998).

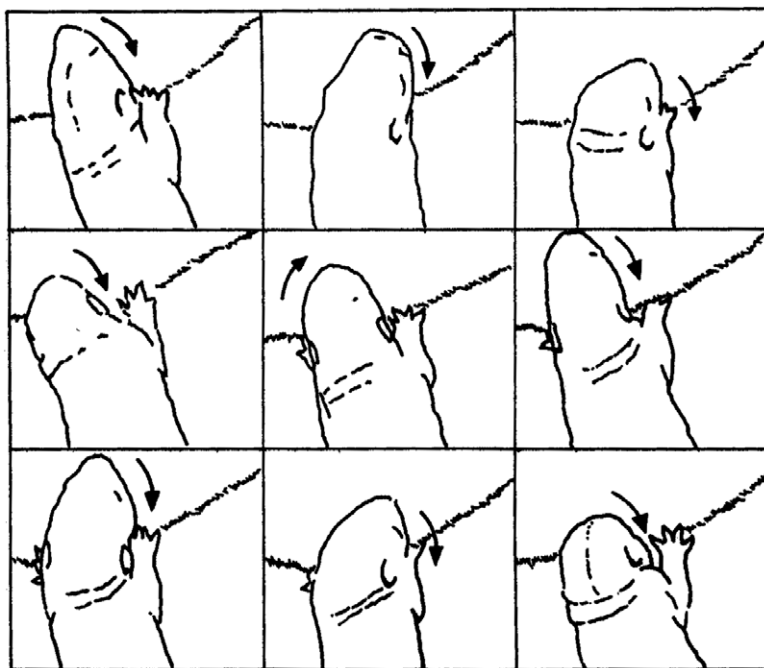


Fig. 4. Scanning movements of a 7-day-old rat pup in the process of locating a nipple of an anesthetized dam. Each panel represents a single frame from a videorecording (Adapted from Pedersen and Blass, 1981).

A related challenge to accurate analysis of the effects of gravity on development is separating effects of gravity on mothers vs. offspring. Because the maternal–fetal system is so highly intertwined in mammals, differentiation among these “direct” and “indirect” effects of gravity on the developing young is a critical, yet extremely difficult task. As we have previously discussed (Alberts and Ronca, 1999), “direct” effects of gravity are those that operate through a primary relation with the recipient organism, tissue or cell. In this case, applications of fractional g -loads would be expected to produce fractional differences from the $1-g$ phenotype. In contrast, “indirect” effects of gravity are those expressed through avenues of the mammalian system. For example, if mothers cannot stabilize their bodies in the nursing posture or if pups cannot retain metabolic heat because their huddling behavior is disrupted (see below), the altered growth effect would clearly be an indirect consequence of weightlessness.

The huddle as habitat

As the pups develop, the mother begins to make more frequent and longer excursions from the nest. As this occurs there is, in effect, a habitat shift.

Compared to the birth transition, this one is gradual and subtle, but the effect is profound. The huddle of littermates becomes habitat (Fig. 1C) and the pups interact in important and adaptive ways.

There is great thermal significance to the huddle (Alberts, 1978). Though their thermogenic capabilities are limited and their insulation is meager, pups can generate metabolic heat, mostly through activation of brown adipose tissue, a uniquely mammalian specialization. By huddling with their littermates there is less heat-losing surface area exposed to the environment and there is the opportunity for conductive heat exchanges. Pup behavior augments this. A huddle is not a pile of passive bodies. Pups move and exchange positions frequently. In some ways, the huddle behaves like a single organism and displays “group behavioral regulation” whereby the huddle regulates its own surface: mass ratio and hence its thermal properties (Alberts, 1978). The key to this feat is the behavior of individual pups, some of which involves diving down and moving up through the mass of bodies in a nest. Other behavioral factors include movements that bring pups together and maintain contact. Responding to the activity state of other pups is also key to the development of a more integrated, responsive group behavior (Schank and Alberts, 1997). The huddle becomes habitat, exclusive of the dam, for significant portions of each day beginning around day 5. It gains in prominence through about day 15, when longer and more frequent egressions from the nest begin.

In addition to the important thermal benefits of huddling, pups also derive cutaneous and proprioceptive stimuli from the contact behavior. This is probably a significant feature of the huddle as habitat. Rats are considered a “contact species” because even their adult social behavior is dominated by physical contact among conspecifics.

Anticipating the gravitational aspects of the huddle as habitat

Hardware design will again be critical to the science. Huddling should be enabled, but not necessarily forced. Ideally, the environment will allow self-regulation by individuals and the group.

It is possible, based on observations such as those made during parabolic flight studies, that weightless pups will react to weightlessness by becoming even more responsive to tactile cues than they are on Earth. That is, during the “0-g” phases of parabolic flight, pups tended to grab and tenaciously hold onto grids, wires, each other, even their own tail (Alberts, unpublished observations). If such tendencies persisted during continuous exposures to microgravity, the pups would create a novel tactile environment for themselves and this would require incorporation into the subsequent interpretation of outcomes.

Figure 5 is from a study (conducted at 1-g) of pup movements in a huddle that was contained in a bowl-shaped nest. Gravity was at work, of course. But so were the pups. The arrows superimposed on the drawings depict



Fig. 5. Drawings of a huddle of 10-day-old rat pups in a concave nest. The arrows depict the direction of “pup flow” when individuals burrow and dive into the group.

the movements of the pups as they burrowed *down* into the huddle. Their individual movements in the huddle create a phenomenon termed “pup flow” and this changes systematically with changes in the micro-environment. Under the conditions used in this study, the pups’ behavior was motivated by thermal conditions, for when the nest temperature was increased, the warmed pups reversed directions and pup flow was *up*. How will this be executed in different gravities? How will the resulting self-stimulation of the musculature, proprioception, and the vestibular system be expressed later in life? Such questions, we think will be key to developmental insights emerging from this habitat.

Some of the pups in Neurolab studies flew during the developmental phase that we are discussing here. The conditions experienced by the Neurolab pups launched on postnatal day 8 might have potential associations with a number of postnatal impairments or delays. For example, undernourishment in early life causes significant delays in physical growth of the young and long-lasting morphological changes, particularly in brain areas undergoing cell proliferation (e.g., [Fish and Winick, 1969](#)). Reduced neocortical dendrogenesis, number of dendritic spines, and reduced synapse-to-neuron ratio have been reported following neonatal malnourishment. These changes are frequently correlated with reflexive and long-term behavioral abnormalities, including aberrant locomotor patterns (see [Ronca, 2003](#) for further discussion).

In an effort to identify critical periods in peripheral vestibular system development, plastic changes in the organization of the vestibular efferent network of the rat utricle were studied in the postnatal rats launched on day 9 of the Neurolab flight ([Dememes *et al.*, 2001](#)). Immunofluorescence experiments were performed with a specific biochemical marker of the efferent system, the calcitonin gene-related peptide (CGRP). The utricles were analyzed by confocal microscopy. Maturation of the vestibular efferent system was similar in space flight and control rats. The absence of changes in the development of vestibular efferents is encouraging in view of the dramatically reduced body weights of the pups studied.

The coterie as habitat

Though the nest diminishes as the site for behavioral interactions beginning around day 15–19, the pups remain highly social and interactive with each other and with the dam, as well as with other conspecifics in a colony or colony-like setting. The pups are on the verge of weaning at this stage, i.e., they will begin to eat and drink independently and gradually diminish suckling and milk intake. At this stage, interactions with littermates continue but they occur outside the nest. In a real sense, the social cohort or coterie becomes habitat. Huddling, mutual grooming, and play fighting are observed in a variety of situations. Feeding is a social behavior, and much is known about the onset of food intake and the formation of dietary preferences and other socially mediated choices (Galef, 1981).

Just as we noted that the suckling pup was exquisitely equipped to utilize milk as diet, the weanling demonstrates corresponding adaptations to a diet of solid food, usually higher in carbohydrates and protein than was mother's milk. Figure 6 depicts some of the dramatic changes that enable the weanling to leave milk, eat food, and maintain a trajectory of growth and development. We present this graph as a representation of the multileveled adaptive changes that the developing pup displays. These changes in digestive enzymes are beautifully coordinated with changes in morphology, sensory function, and behavior (e.g., Alberts, 1994).

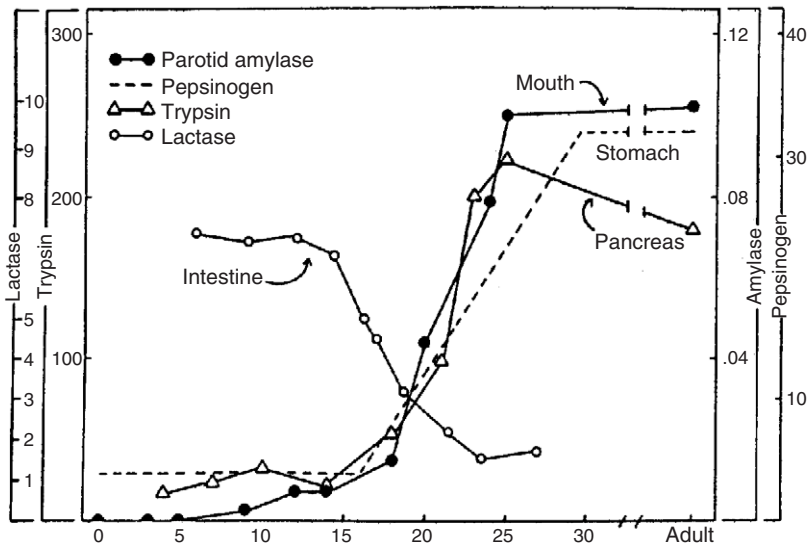


Fig. 6. An overview of selected physiological developments that enable adaptation to suckling (intestinal lactase) and to the utilization of solid food, as measured in rat intestine, mouth, stomach, and pancreas (from Alberts, 1994).

During this phase of life, pups are establishing a more independent existence, though they remain highly social and contactful, at least on Earth. Sexual maturation is proceeding and, in the course of such changes, sexual differences begin to emerge. These are seen in physical developments as well as in behaviors, including grooming and play.

The coterie and gravity

Again, the kinds of habitats in which animals develop are likely to contribute to the outcomes seen. A deep understanding of the many dimensions of adaptation to the social and physical habitat during this phase will be important in our ability to understand gravitational adaptations.

Bone and muscle develop importantly during this phase of growth and maturation. What the animals do in different gravitational fields, as well as how much they do it, will be key to data interpretation.

It is especially enticing to take note of a number of findings from the Neurolab mission derived from pups flown in the AEMs at ages that involved the coterie as habitat. A sample of these are noted herein. It is valuable to keep in mind the benefits that will be derived from having at hand a full view of what these animals experienced in the coterie, as a basis of understanding the outcomes.

The myosin heavy chain (MHC) molecule is the principal structure and regulatory protein that serves as the molecular motor to control the intrinsic contractile properties of muscle (Adams *et al.*, 2000). The separate and combined effects of space flight and thyroid deficiency on myosin heavy chain (MHC) gene expression (protein and mRNA) were examined in muscles of the Neurolab pups. It was found that space flight markedly reduced the expression of the slow, type I MHC gene by approximately 55%, whereas expression of the fast IIX and IIB MHCs in antigravity skeletal muscles was enhanced. In fast muscles, space flight caused subtle increases in the fast IIB MHC relative to the other adult MHCs.

Central pathways for oxytocin and vasopressin have been implicated in the neurobiology of anxiety and social behaviors. Garcia-Ovejero *et al.* (2001) studied oxytocinergic and vasopressinergic magnocellular hypothalamic neurons of prepubertal rats of the 15-day-old rat pups flown on the Neurolab mission either at landing or 18 weeks postlanding. Enhanced transcriptional and biosynthetic activity was observed in magnocellular supraoptic neurons of flight animals on the day of landing as compared to control rats, including increased c-Fos expression, enlarged nucleoli and cytoplasm, and increased volume in the neuronal perikaryon of mitochondriae, endoplasmic reticulum, Golgi apparatus, lysosomes, and cytoplasmic inclusions (nematosomes). Vasopressin levels, cytoplasmic volume and c-Fos expression returned to control levels by 18 weeks after landing, whereas other changes did not normalize. Together, the results of this study suggest that space flight during the

preweaning period may induce irreversible modifications in the regulation of oxytocinergic neurons. Central pathways for oxytocin and vasopressin have been implicated in the neurobiology of anxiety and social behaviors.

A study of the postnatal day 15 pups flown on the Neurolab mission suggests that development in microgravity leads to a constellation of interesting changes in the number and morphology of cortical synapses and does so in a laminar-specific manner (DeFelipe *et al.*, 2002). In the layers II/III and Va, the synaptic cross-sectional lengths were significantly larger in flight animals than in ground control animals. Flight animals also showed significantly lower synaptic densities in layers II/III, IV, and Va. The greatest difference was found in layer II/III, where there was a difference of 344 million synapses per mm^3 (15.6% decrease). After a four-month period of re-adaptation, some changes disappeared (i.e., the alterations were transient), while conversely, some new differences also appeared. For example, significant differences in synaptic density in layers II/III and Va after re-adaptation were no longer observed, whereas in layer IV the density of synapses increased notably in flight animals (a difference of 185 million synapses per mm^3 or 13.4%). In addition, all the changes were in asymmetrical synapses, known to be excitatory. These results suggest that gravity may be an important environmental parameter for normal cortical synaptogenesis.

Temple *et al.* (2002) studied spatial learning in the postnatal day 9 or 15 Neurolab pups using several different tasks. Performance and search strategies evaluated for each task revealed remarkably few differences between the flight groups and their Earth-bound controls at both ages. Together, these data suggest that development in an environment without gravity has minimal long-term impact on spatial learning and memory abilities. Nevertheless, we must more fully understand the ways and rates at which adaptation to micro-*g* and re-adaptation to 1-*g* occur before we can fully interpret such findings.

Developmental biology as a core discipline for space biology and biomedicine

Throughout the present chapter we have noted, even celebrated, patterns of development that are unique to and universal among mammals. From the standpoint of evolutionary biology, such commonality of process represents the common ancestry shared by contemporary mammalian species. It reinforces the unity and continuity among mammalian forms and is the foundation for cross-species generalizations and detailed comparisons of differences. From the standpoint of fundamental biology and biomedicine, because mammals share so many biological processes, species such as the Norway rat are valid models of human function and disease. This has been explored and validated in ground-based science; the principles apply equally well to contemporary concerns in the space life sciences.

How is rodent development pertinent to space biology and space biomedicine? We believe that rigorous study of the gravitational biology of

vertebrate development is a valuable pursuit, directly and perhaps uniquely relevant to some of the most pressing issues of astronaut well being in an era of extended missions and possible space exploration.

First, *adaptation* is the most general and pervasive question regarding human health during long-term missions. Fundamentally, as we have shown, *development is adaptation*. Each insight gained into the roles of gravity in development is an increment of understanding better the grand issue of adaptation to the space environment. Among potential advantages of studying space adaptation via development rather than adaptation in adults, are rapidity and magnification. To be accurate, studies of adults over time are also developmental studies, for there really is no fixed or “final” adult stage. Nevertheless, changes during adulthood typically proceed more slowly and subtly than during phases of rapid growth and reorganization. Generally, during phases of rapid growth and reorganization, such as the postnatal phase from birth to weaning in mammals, the changes are rapid and dramatic. The underlying processes in this phase of life are the same as those at other phases, but they occur rapidly and are often expressed more dramatically (magnification). We have endeavored to show how the somatic plasticity evident during development can be understood as adaptive change. Many of these processes are gravity sensitive. Thus, we can conclude that developmental preparations are relevant to and efficient for space studies.

Another way in which some investigators use developmental analysis is as a window into understanding how a system is “built” or assembled during maturation. This approach can be used to gain insight into how complex systems are organized and how it may be that some components are differentially sensitive to perturbation. Again, this is an adaptive issue and developmental analyses can be the key that unlocks the passageway to deeper understanding.

With such considerations in mind, we see a fruitful and profitable future for space research that includes developmental analysis in its agenda. To the extent that such studies have been supported in the past, the results have been rewarding and varied. Knowledge and expertise is accumulating and the pace at which new knowledge can be generated will increase further with continued support.

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