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NASA-Ames Coop. Agreement NCC 2 862
Development of sensory receptors in skeletal muscle

Rat Gestation during Space Flight: Outcomes for Dams and Their Offspring Born after Return to Earth

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Abstract—Sprague-Dawley rats were studied to learn whether gestation in the near-zero gravity, high radiation environment of space impacts selected mammalian postnatal events. Ten rats spent days nine to twenty of pregnancy aboard the space shuttle orbiter *Atlantis* (STS-66). Their movement was studied shortly after return to Earth; subsequently, several of their offspring were cross-fostered and examined through postnatal day 81 (P81) for whole body growth and somatic motor development. Values for the flight animals were compared to ground-based control groups. Relative to controls, the pregnant flight rats showed a marked paucity of locomotion during the first few hours after returning to Earth. There was greater likelihood of perinatal morbidity for the offspring of flight dams when compared to the control groups. Whole body weight of surviving offspring, averaged for each group separately, showed typical sigmoidal growth curves when plotted against postnatal age. The flight group for our study had a larger ratio of female to male pups, and that was sufficient to account for the lower average daily weight gained by the flight animals when compared to the control groups. Walking was universally achieved by P13 and preceded eye opening, which was complete in all pups by P17. Thus, both of these developmental horizons were attained on schedule in the flight as well as the control rats. Characteristic changes were observed in hind limb step length and gait width as the pups grew. These patterns occurred at the same time in each group of rats. Therefore, prenatal space flight from days nine to twenty of gestation did not interfere with the establishment of normal patterns for hind paw placement during walking.
Key words—space shuttle, mammalian development, microgravity, weight gain, eyelid disjunction, locomotion

Introduction

THERE ARE THEORETICAL and practical reasons for wanting to understand how living things accomodate to space flight conditions. An example of the former is that life on Earth has inherently co-existed with gravitational effects, so learning how gravity influences all stages of life in an organism is an intriguing endeavor. Practically speaking, we should be aware of possible adverse effects that conditions of space flight may have as we explore the frontier of outer space. The history of space exploration as regards effects on development has progressed through various stages. Microbial life, plants and various animals—including fish among the vertebrates (Ijiri, 1995)—have been shown to be capable of

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Integrative Physiological and Behavioral Science, October–December 1997, Vol. 32, No. 4, 322–342.

reproducing and developing successfully in outer space (Miquel & Souza, 1991). Mammalian reproduction there becomes more probable as men and women live away from Earth for longer periods of time.

Investigations of space flight effects on mammalian development have been few (see reviews by Miquel et al., 1991; Wolgemuth & Murashov, 1995). It remains to be determined whether space flights encompassing longer periods of gestation affect embryogenesis and postnatal development. Two unusual conditions extant during low Earth orbit space flight—as compared to existence within the biosphere—are exposure to a decreased gravitational field (microgravity) and increased radiation. Ground-based studies have shown that exposure to analogous environments alters postnatal development. Examples include findings that: 1) hypergravity during rodent gestation decreases the number of live births per litter and lowers survival rates of the offspring (Johnson & Oyama, 1995; Oyama et al., 1985), and 2) locomotor impairment results in rats after sublethal X-irradiation received while *in utero* (Mullenix et al., 1975).

Studies of mammalian development in space began with the Soviet Cosmos biosatellite experiments. The 18.5 day Cosmos 1129 mission (September 25, 1979, launch) investigated the effects of space flight on fertilization, implantation, embryogenesis, and postflight reproductive function in rats. Although no animals were born as a result of conception among the flight rats (or among the ground-based synchronous controls, according to Keefe, 1985), there was equivocal evidence of ovulation and pregnancy, suggesting that gestational events may have gone awry (Serova and Denisova, 1982). Outcomes of tests for postflight reproductive status (flight males mated with control females) were that offspring conceived within a week after flight had some transient physiologic abnormalities at birth, while those conceived months later by the same adults did not (Serova and Denisova, 1982). However, those postflight matings did not result in a difference from controls at either of the two time periods when end points were number of newborns and birth weight (Serova and Denisova, 1982). Experiments conducted on Cosmos 1514 (December 14, 1983, launch) focused on mid-gestational development in rat fetuses. The results showed that, while space flight conditions may have altered the rate of ontogenesis during the fetal stage—affecting weight and attrition of newborns—the overall postnatal development of surviving offspring was generally similar to that of controls (Alberts et al., 1986; Serova et al., 1984). That mission lasted five gestational days (G13–G18, where G0 is the day of conception); it was the first demonstration that mammalian fetal development in microgravity was possible. In the decade after the Cosmos 1514 mission there were no studies in outer space involving the development of placental mammals.

The National Institutes of Health–Rodent 1 (NIH.R1) payload addressed the issue of whether the conditions of space flight during most of the last half of gestation would affect development of rats while *in utero* and subsequent to their birth. NIH.R1 was aboard mission 66 of the Space Transportation System (STS-66), commonly known as the space shuttle, launched November 1994 by the National Aeronautics and Space Administration (NASA). Offspring had records made of their body weights, times of eyelid disjunction and initiation of locomotion, and progression of walking. Play behavior and reproductive function of the offspring were also assessed, as was the immediate postflight locomotor state of the dams. Some of these findings have been reported in abstract form (DeSantis et al., 1995; Parkman et al., 1995; Wong et al., 1995).

TABLE 1
Experimental Variables Experienced by Rat Dams

Group	*Space Flight*	*AEM Housing*	*Surgery*[a]	*None*
Flight	X	X	X (h and l)	
Synchronous control		X	X (h and l)	
Vivarium A control			X (h)	
Vivarium B control				X

Note: [a] hysterectomy (h), laparotomy (l).

Methods

Sprague-Dawley rats, *Rattus norvegicus* (Taconic Farms, Inc.), were studied. All surgical procedures on the dams were done according to National Institutes of Health Guidelines; individual experimental protocols were approved by Institutional Animal Care and Use Committees at NASA-Ames Research Center, Kennedy Space Center, and University of Idaho. Prior, ground-based assessment of the surgical, housing, and selected other protocols provided the basis for the experimental design subsequently used for the actual flight study (Alberts et al.,1996).

Dams

Experimental design. Ten pregnant dams were equally divided and placed into two animal enclosure modules (AEMs) (Greenawalt, 1993) where they experienced 10^{-3}g (1g = Earth gravity) for eleven days, from gestational days nine through twenty (G9–20, where G1 is the day of conception). An AEM is a living chamber that allows for continual food and water availability and waste removal from the air without human intrusion into the unit. The AEMs were fitted into middeck lockers aboard the space shuttle *Atlantis* and launched into orbit around Earth on November 3, 1994 (~noon EST) from the Kennedy Space Center (KSC), Florida. Three groups of ground control dams were maintained at KSC. The synchronous controls (n=10) were also split between two AEMs but had their pregnancies offset 24 hours later than the flight dams, which enabled the temperature (28–30°C), humidity (25–75 percent), and light:dark cycle (12:12) to be matched to those conditions experienced by the flight group. As was the case for flight dams, the AEMs housing the synchronous controls remained closed throughout days 9–20 of gestation. However, neither acoustic, vibrational nor gravitational stimuli associated with launch and recovery of the shuttle were simulated for the synchronous control group. The other two control groups were kept in standard Plexiglas™ cages (1 dam/cage) maintained under routine vivarium conditions and are thus designated as vivarium controls. One set, (A), of vivarium control dams (n=12) had a unilateral hysterectomy done on G20 to remove fetuses for other studies; that was also the case for flight and synchronous control dams. But, unlike those latter two groups, no laparotomy was done at G7 on vivarium A rats to confirm pregnancy and estimate the number of fetuses. The other set, (B), of vivarium controls (n=12) had no surgery prior to delivery of their young. Thus, the four groups of rats differed in the interventions they experienced, as shown in Table 1.

Video recordings. Postflight videos of flight dams were taken within three hours after space shuttle orbiter landing at Dryden Flight Research Facility (DFRF), California on November 14, 1994 (~10:30 a.m. EST). Recordings of synchronous control dams housed

at KSC, Florida, were obtained at an equivalent time of day, but twenty-four hours later than the flight dams so that the same day of pregnancy was compared. Each dam was removed from her home cage and filmed for between one and two minutes while in a clear rectangular container (44 cm long x 29 cm wide x 29 cm high), which allowed simultaneous video recording from the top and side. Levels of somatic motor behaviors exhibited by the dams—exploring their surroundings, rearing up onto their hindlimbs, and grooming—were ascertained from the videotapes by three observers independently. Counts were averaged for each rat, and the average number of times per minute all dams performed each of the three activities was compared between the two groups.

Delivery of offspring. After the video recordings, unilateral hysterectomies were performed on the flight, synchronous and vivarium A dams to remove G20 fetuses for separate studies. They were then housed individually in the vivarium until they began delivering pups from the remaining uterine horn two or three days later. Two flight dams that did not deliver vaginally by midday of G23 had Cesarean sections to remove their pups. Two dams from control groups (synchronous and vivarium A) also had Cesarean deliveries for the same reason.

Pups

Experimental design. The offspring we tested were obtained postflight from the experimental or flight dams and from those of each control group. Based on the overall design of the experiment, our study had been assigned certain pups from each of the dams, (i.e., those at positions #iv and #ix in the remaining uterine horn, where each fetus in that horn was identified in sequence, starting nearest the body of the uterus). We had available initially the following number of pups in each group: flight = 10; synchronous control = 10; vivarium A control = 11; and vivarium B control = 26.

A numbering code, which was in sequence with birth chronology, was tattooed on the day of birth—designated postnatal day one (P1)—into the back skin of each pup used in the study. Different colors of ink coded the group to which each pup belonged (flight-black, synchronous control-red, vivarium A control-blue, vivarium B control-green).

Because the dams were sacrificed shortly after parturition for other's studies, the pups to be reported on here were fostered into litters that had been born within the previous twelve hours. The total number of pups in each cross-fostered litter was ten, and not more than half of that total were foster pups. The remaining pups in these litters were the foster dams' own offspring; their tattooed number was coded with orange ink. None of the flight pups were mixed with any of the synchronous and vivarium control pups in foster litters because of the geographic separation of launch (all ground-based control pups) and recovery (flight pups) sites. There often were mixtures of pups from the various control groups into a foster litter. The foster litters from DFRF and KSC (13 litters total) were shipped by air to the University of Idaho on P3 and P4. The size of each foster litter was culled to nine pups per litter on P6 because two vivarium control pups—one each from the A and B groups—did not survive cross-fostering. Except for the two litters containing those two vivarium pups, the rats culled were the foster dams' own. Foster litters were maintained until weaning, at which time the foster dams' own pups were sacrificed, and the flight and control rats were housed with members of the same sex. Cages were kept in a controlled environment (20°C, ~78 percent relative humidity and a 12:12 hour light:dark cycle). The animals were allowed food and water *ad libitum*.

Gender assessment. The sex of each rat born alive was recorded after examining the

external genitalia at the time of birth. For the pups of this study, which were sacrificed when adults, the animal's gender was verified by inspection of internal genital structure.

Postnatal weight. At birth each pup was weighed to the nearest 0.01 gm; that value was recorded as the birth weight. From P1 through P30, each animal was weighed to the nearest 0.1 gm at about the same time daily. Between P30 and P80, body mass was measured every other day.

Initiation of walking and of eye opening. Beginning by P2, pups from each group were gently placed ventral side down on a smooth, flat, metal surface at ambient room temperature. They were observed for 5 minutes daily until walking and eye opening were attained by all members of the litter. We defined walking as the ability of a rat to support its body weight off the floor on all four limbs and to move forward at least one body length. If the rat pup did not walk within the observation period it was given a score of 0. If it did walk within the time limit it was scored as 2. If the animal either did not move forward one body length when it had its body lifted from the surface or, as was more often the case, it moved forward at least a body length but with its ventral surface touching or bouncing against the walking plane, its score was 1.

Eye opening was defined as the separation of the upper and lower eyelids sufficiently for an observer to see any part of the rat's eyeball through the resulting palpebral fissure. Scores for eye opening were: 0 = neither eye open, 1 = only one eye open, and 2 = both eyes open.

Hind limb use during walking. Use of hind limbs during locomotion was assessed from paw print records (Mullenix et al., 1975; Rushton et al., 1963). When a pup had reached the true walking stage, by P10 for most pups, hind limb walking patterns were recorded in a confined runway. Three runways were used to accommodate the growth of the rats. All were constant width (8.9 cm); the length was varied (30, 45.7, or 61.0 cm) so that it was at least four times an animal's body length. At the far end of the runway there was a darkened box with food. After two to three conditioning trials, the plantar surfaces of the hind paws were coated with removable ink (Pelikan 4001 plus 20 percent Triton X-100 detergent) and then the rat walked the distance of the runway along a sheet of absorbent paper. Digit and paw pad positions imprinted on the paper (Figure 1). After testing, excess ink was rinsed from the animal with water, and the rat was dried with paper toweling and returned to its cage.

The ink prints were measured using a digitizing tablet (HIPAD), coupled to a computer (Apple IIe) loaded with morphometric software (Bioquant). Two measures of hind limb use during locomotion were analyzed: step length and gait width. Step length or stride is the distance on the walking surface covered by the same hind limb (left or right) during successive steps; gait width or spread is the distance between the left and right hind limbs as the rat walks. Distances between contralateral and ipsilateral paw prints from successive step cycles were measured for each rat. An average value for stride was obtained from the ipsilateral distances for each rat on each day of testing. A mean value for spread was calculated from the Pythagorean theorem ($c^2 = a^2 + b^2$, where b represents gait width) by applying the average measurements of contralateral (c) and ipsilateral (a) step lengths. Finally, average values for stride and spread were obtained for each group of rats on each day of testing. From days P10 through P30, hind limb walking patterns were obtained from animals every other day. From P30 through P81, measurements were collected every third day. All flight and synchronous control rats and a sample (n=10) of the vivarium controls, A plus B, had walking patterns recorded for each time point.

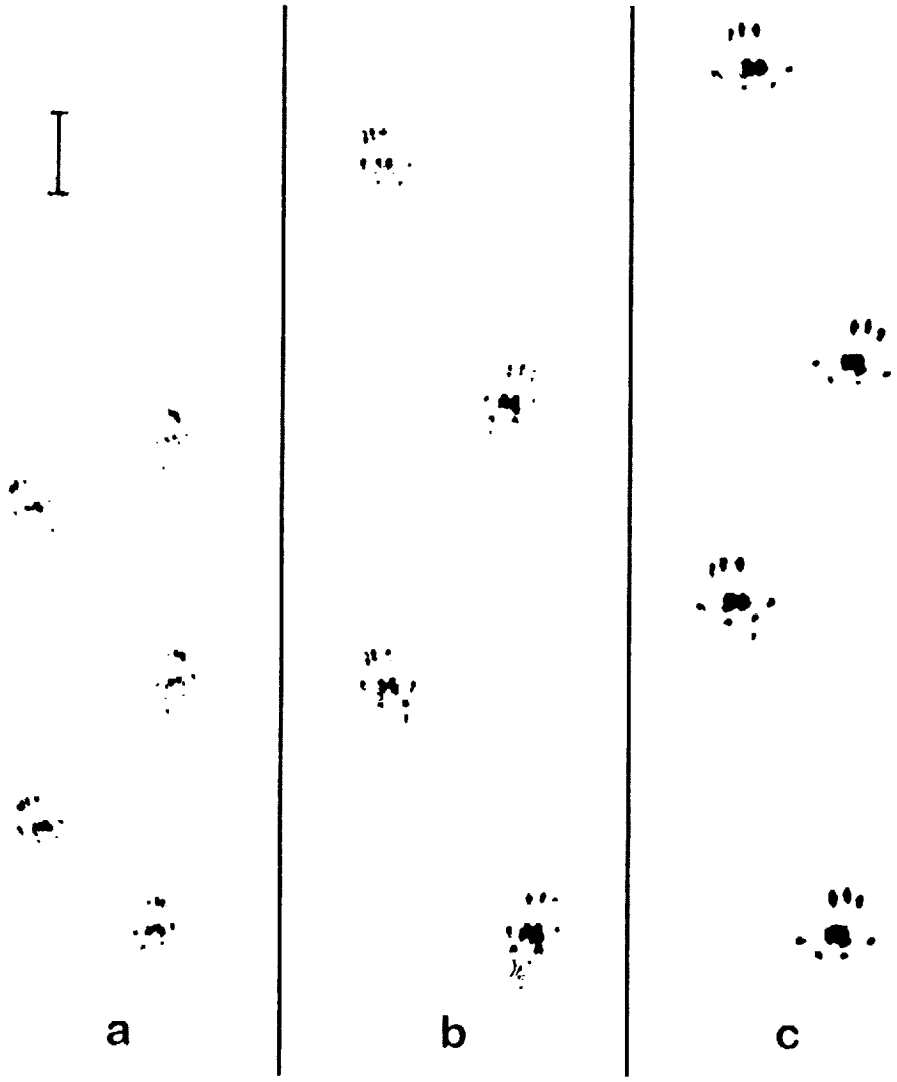

FIG. 1. Hind paw walking prints from three rats of different age, sex, and group: (a) P22 flight male, (b) P42 vivarium B female, (c) >P80 foster dam. Walking is in the direction towards the top of the figure; calibration equals 2cm and applies to all panels. The obvious increase in step length with relatively little increase in gait width is a characteristic change in locomotion that directly correlates with age rather than with the other two variables (see Figure 4).

Statistical Analysis

Student's T-test was used to compare the amount of movement of the flight versus synchronous control dams videotaped upon their return to Earth.

Data obtained from the pups were analyzed using a variety of statistical tests. A repeated measures analysis of variance (ANOVA) was used to evaluate differences in body

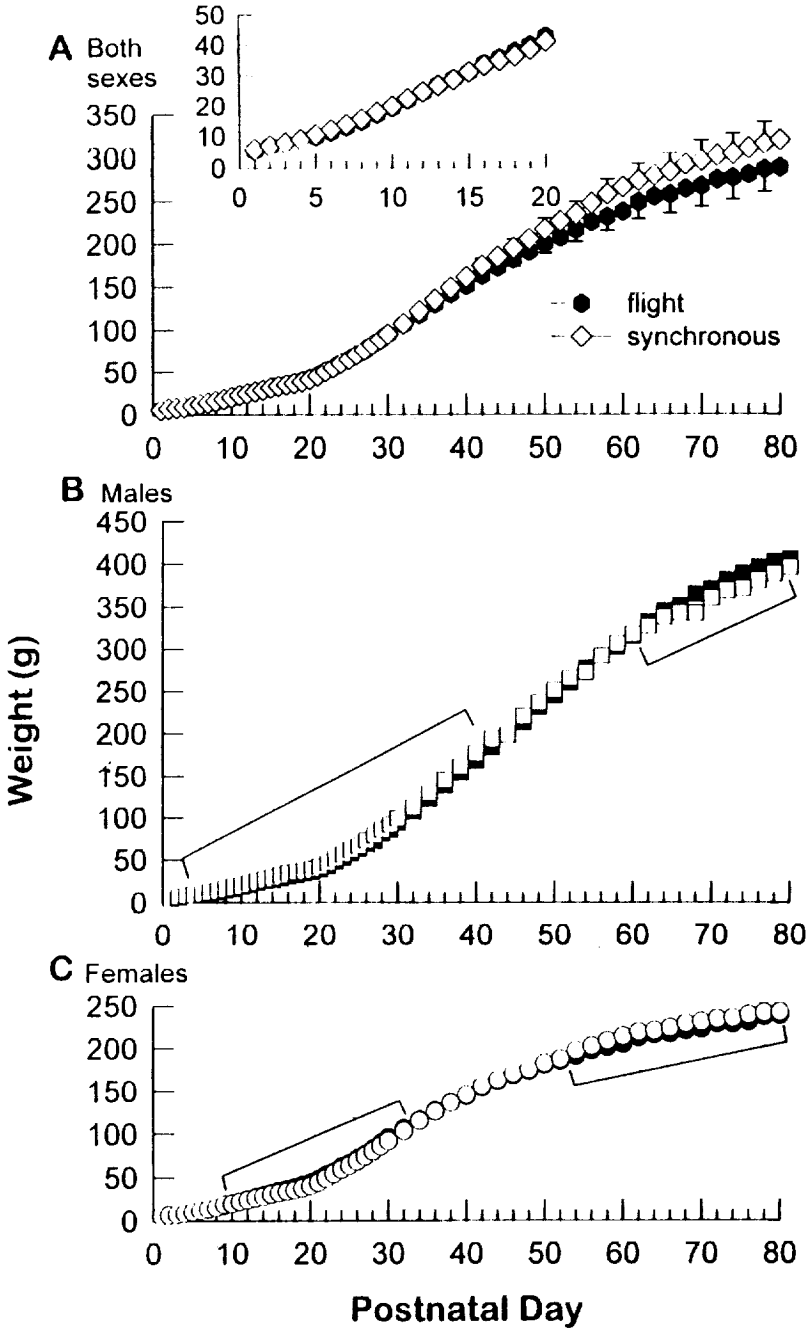

FIG. 2. Body weight comparisons for flight (filled symbols) versus synchronous control (open symbols) rats from birth through P80. A) Group average weight (+/- SE) of flight (hexagon) versus synchronous control (diamond) animals. Inset shows detail for P1-20. A separation between flight and synchronous control begins at about P36 and accentuates through P80. Group average weights for males (B) and females (C) show that there is no difference between the experimental and control groups when genders are considered separately. Note phenomenon of oscillatory growth (brackets) whereby a group whose average weight initially is slightly but consistently above (or below) that of the other, will have the opposite relationship at a later age.

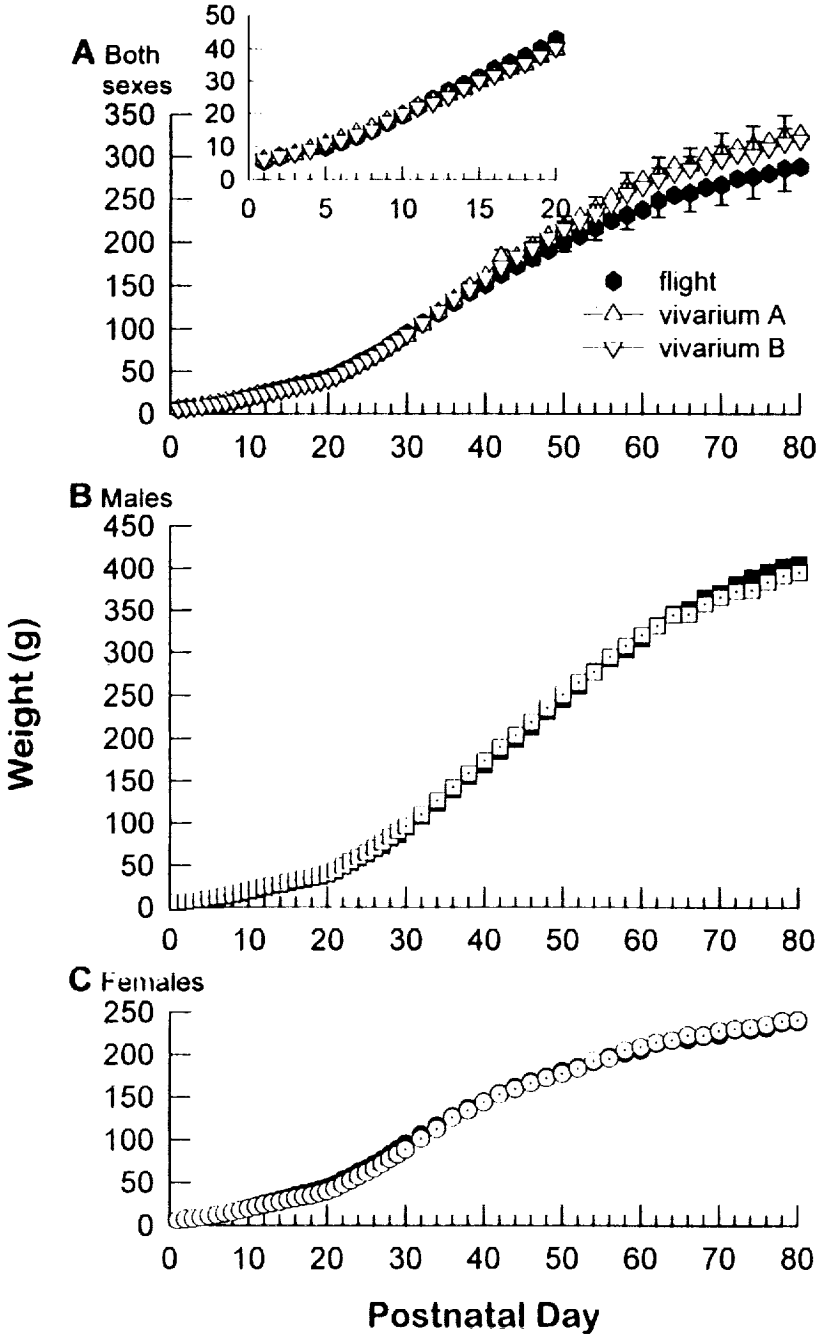

FIG. 3. Body weight comparisons for flight (filled symbols) versus vivarium control (open symbols) rats from birth through P80. A) Group average weight (+/- SE) of flight (hexagon) versus vivarium A (up triangle) and vivarium B (down triangle) animals. Inset shows detail for P1-20. Flight rats as a group consistently weighed less than either set of vivarium controls as the animals grew after the first postnatal month. When males (B) and females (C) are evaluated separately, however, there is no difference between the experimental and control (combined vivarium groups shown as open symbols with center dot) groups. Oscillatory growth is evident as in Figure 1.

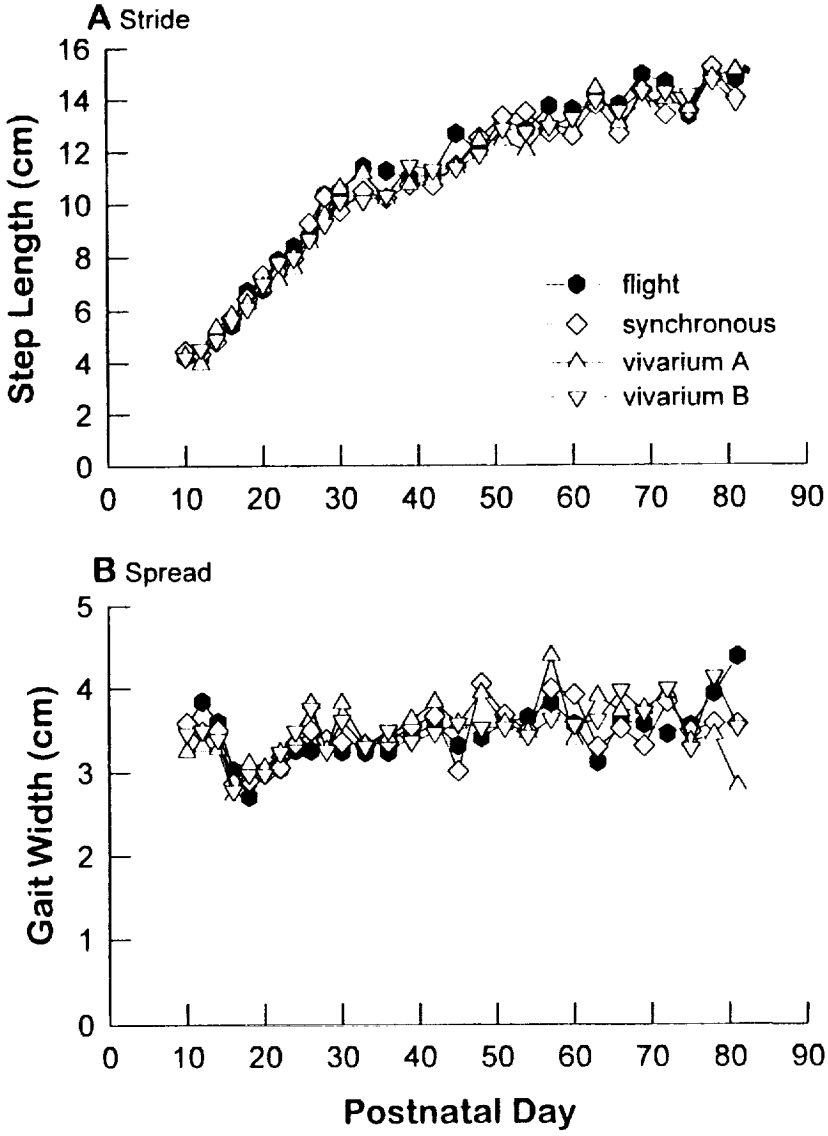

FIG. 4. Progression of hind limb stride and spread during walking in flight group (filled hexagon) and each control group (open symbols: synchronous-diamond, vivarium A-up triangle, vivarium B-down triangle) measured from paw prints. Average step length (A) increased by 10cm in all groups over the time period studied, whereas average gait width (B) increased by about 1cm.

weight and hind limb use during walking. We expressed variation about the average value as +/- either the standard deviation (SD) or the standard error (SE) of the arithmetic mean. Treatment effects were tested for statistical significance ($p < 0.05$) using "Fisher's protected least significant difference (LSD) for least squares means (LSM)." Because step length had not been measured on identical vivarium control rats on each day, split plot

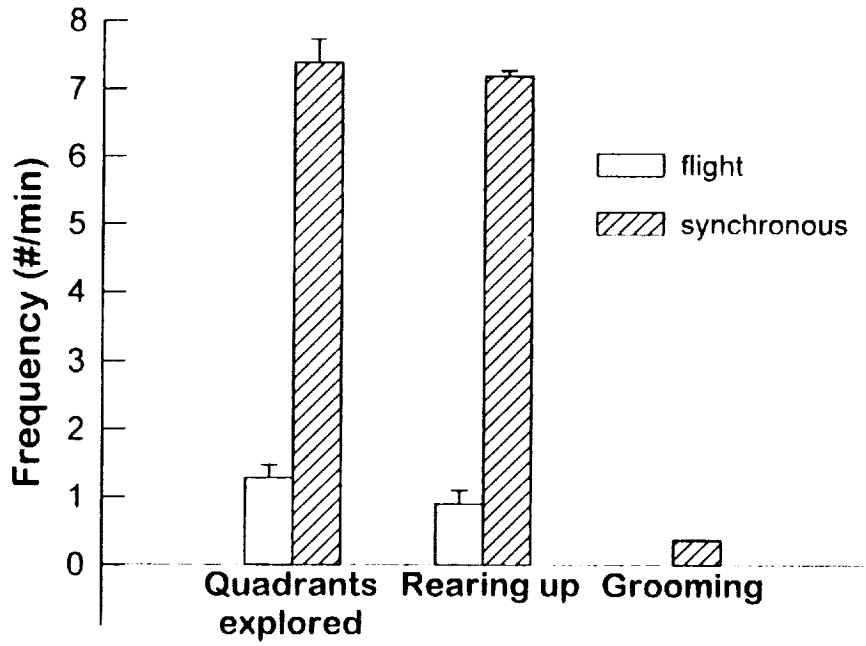

FIG. 5. Average frequency of three behaviors by pregnant rats within three hours of return to Earth gravity. Flight dams (open bars) were far less active than gravid synchronous controls (hatched bars). Values are mean +/- SE. Differences are statistically significant.

general linear model for unequal subclass numbers was fit to data on locomotion. The whole plot treatments were group and sex. Rats were nested within the group-sex subclasses. The whole plot error term was used to test sex and group main effects and the "sex by group" interaction. The main effect of days and interaction of: 1) "days by group," 2) "days by sex," and 3) "days by group by sex" were tested using the split plot error term. The overall time period from P1 to P81 was subdivided into three or four epochs when body weight and locomotion were evaluated statistically. Selection of these epochs was based on places where the vivarium control data curves showed changes in their slopes; those epochs were different for body weight (Figures 2 and 3), step length (Figure 4A) and gait width (Figure 4B).

Initiation of eye opening and walking was tested using Probit analysis for "Effective Day_{50}" (ED_{50}). The ED_{50} is that time, calculated mathematically from the data, when achievement reaches half of the way to full criterion level.

The critical comparison for identifying differences attributable to space flight is between the flight and synchronous control groups; they were the most closely matched in all respects except for experiencing those variables specifically associated with space flight (Table 1). Additional comparison with the two vivarium control groups permits an evaluation of how other variables in the experimental design, such as housing of the rats and surgical procedures done on them, may have affected the outcomes (Table 1).

TABLE 2
Number, Sex, and Perinatal Mortality of Rat Pups[a]

Group[b]	*Female*	*Male*	*Female / Male*	*Dead-Pups*[c] *[#]*	*[%]*	*Total Pups*
Flight (n=10)	7 [28]	3 [30]	2.3 [0.9]	—[4]	—[6.5]	10 [62]
Synchronous control (n=10)	5 [24]	5 [33]	1.0 [0.7]	—[1]	—[1.7]	10 [58]
Vivarium A control[d,e] (n=11)	5 [30]	6 [32]	0.8 [0.9]	—[0]	—[0.0]	11 [62]
Vivarium B control[e,f] (n=12)	14 [72]	12 [63]	1.2 [1.1]	—[1]	—[0.7]	26 [136]

Notes: [a] [bracketed values] are numbers pertaining to all pups delivered from the flight and control dams, including those that were cross-fostered and used in the present study which are given separately as unbracketed values; [b] {n= } gives the number of dams; [c] pups were counted as dead if they were stillborn and/or cannibalized; [d] one additional dam in this group was not pregnant; [e] includes a female pup that died shortly after cross-fostering; [f] whole numbers are approximately double those in each of the other groups since these pups were delivered from both uterine horns (see Methods).

Results

Postflight Observations of Dams

Compared to synchronous control dams, flight animals demonstrated significantly less exploration of surroundings, rearing upright on hind limbs, and grooming in a novel environment (Figure 5). The only rat of this study that was revideotaped at 8 to 10 hours after return to Earth gravity showed a slight increase in movement relative to its behavior within 3 hours after the flight (data not shown).

Sex Ratio and Perinatal Mortality among Offspring

Data for this study were obtained postnatally from the pups listed in Table 2 (unbracketed values). Flight female offspring assigned to be cross-fostered for our study were slightly more than double the number of males; the control groups had more balanced sex ratios. The gender bias in the sample of flight pups we had to study did not reflect the sex ratio for all pups born to the flight dams, which, like the controls, was nearer 1.0 (Table 2, bracketed values).

A second fact notable from Table 2 is that there was higher perinatal mortality among the flight group. Pups known to be stillborn and/or cannibalized (4 of 62) in flight litters came from two dams. They had been in different AEMs, and both gave birth during the night. Other pups in those litters survived; that may have been because they were cross-fostered when it was realized that their siblings were being decimated. Neither of the flight dams that were caught destroying some of their offspring were notably different from others in their group when using other criteria. For example, they were not at the high or low end of the weight range for measures obtained when the rats were loaded into the shuttle, when they were recovered at landing, or just after parturition; nor did either of them have the maximal or minimal litter size among the flight dams (data not shown). Such perinatal loss was less in the synchronous control (1 of 58) and vivarium A and B control (1 of 198) groups. As was the case for the flight group, neither of the two control

TABLE 3
Average Birth Weight of Cross-fostered Rat Pups[a]

Group	*Female*	*Male*	*Female + Male*
Flight	5.80 +/-0.52 (n=7)	5.30 +/-0.51 (n=3)	5.65 +/-0.54* (n=10)
Synchronous control	5.62 +/-0.30 (n=5)	6.27 +/-0.59 (n=5)	5.95 +/-0.56 (n=10)
Vivarium A control	6.03 +/-0.92 (n=5)	6.22 +/-0.60 (n=6)	6.13 +/-0.72 (n=11)
Vivarium B control	5.88 +/-0.66 (n=14)	6.66 +/-0.54 (n=12)	6.24 +/-0.72* (n=26)

Notes: [a] mean +/- SD in grams, n = number of pups; * statistically significant birth weight difference for flight versus vivarium B control when sexes were combined.

dams who had pups die perinatally were at the extremes of body weight and litter size for their respective groups (data not shown).

A final point gleaned from Table 2 is that the average number of pups born per uterine horn was not dramatically different among the groups. The three control groups had nearly equal averages (range 5.6 to 5.8), which was slightly less than that for the flight group (6.2).

Birth Weight

When body mass at parturition for the pups we studied was considered for each sex separately, there were no significant differences between flight and any of the ground control groups (Table 3). Neither was there a significant difference in birth weight between the sexes within any group (Table 3). In each control group, average male birth weight was higher than that for females, but this pattern was reversed in the flight group, in which males weighed less at birth than females (Table 2). When both sexes were considered as a unit, however, there was a statistically significant difference between the flight and vivarium B control groups. Furthermore, average birth weights for group data decreased sequentially from the highest value in the vivarium B controls, next to vivarium A, then synchronous controls and to the lowest value, which was for the flight group (Table 3, right column). The order of arrangement in birth weight was not peculiar to the pups assigned for this study, because the sequence was matched when all the pups born were considered by group (vivarium A and B = 6.24 +/-0.56gm, synchronous = 5.77 +/-0.50gm, flight = 5.60 +/-0.63gm).

Weight Gain

Body weight from P1 to P80 was averaged for each group of rats separately, and in every case showed a sigmoidal curve when plotted against postnatal age (Figures 2A and 3A). Weight gain was steady from birth until weaning (P20), after which a dramatic increase in growth occurred. The flight group's average daily weight remained consistently lower than that of any of the control groups after P36 (Figures 2A and 3A), but not to a statistically significant degree, even when comparisons were made within discrete epochs of time. When the sexes were analyzed separately, there was far less difference in weight among the groups of rats at any postnatal age (Figures 2B and C, 3B and C). However, in

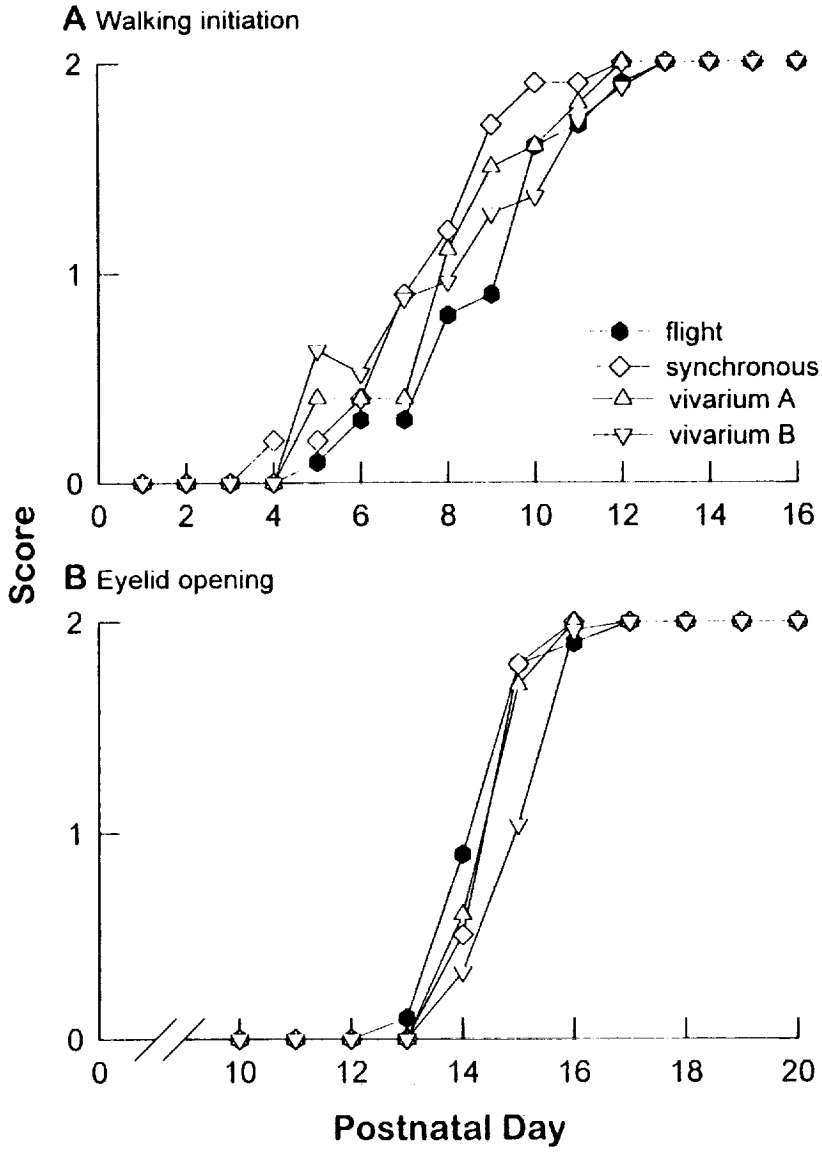

FIG. 6. Scores for initiation of walking (A) and eyelid opening (B) among flight group (filled hexagon) and each control group (open symbols: synchronous-diamond, vivarium A-up triangle, vivarium B-down triangle). As is normally the case for rats, walking occurred in all groups before the eyes opened. Flight pups tended to lag behind the controls in beginning to walk and led them with regard to eyelid opening.

TABLE 4
ED_{50} for Start of Walking and for Eye Opening[a]

Group	*n*[b]	*Walking*[c]	*Eye Opening*[c]
Flight	10	8.28 +/-0.75	14.15 +/-0.31 ⌐ or *
Synchronous control	10	7.13 +/-0.44	14.34 +/-0.25 ⌙
Vivarium A control	10	7.60 +/-0.56	15.01 +/-0.65
Vivarium B control	25	7.49 +/-0.60	15.60 +/-0.87 *

Notes: [a] ED_{50} = calculated postnatal day at which either 50 percent of the rats in a group reached the goals of walking and eye opening, respectively, or 50 percent of a goal was reached by all pups in the group; [b] number of rats tested for each endpoint; [c] ED_{50} +/- 95 percent confidence interval; * statistically significant difference for either flight or synchronous control versus the vivarium B control for eye opening.

each group the weight of females was lower than that of males after P30 (compare Figures 2B vs. C, 3B vs. C); those gender differences were statistically significant.

Initiation of Locomotion

Onset of walking preceded that for eyelid opening in all groups of rats (Figure 6). Some pups in each group had begun walking by P5, and by P13 quadrupedal locomotion had been achieved by all animals (Figure 6A). The flight group lagged slightly behind the synchronous controls (Figure 6A), however none of the differences among groups for ED_{50} were statistically significant by Probit analysis (Table 4).

Eyelid Disjunction

Data for the start and completion of eyelid opening is given in Figure 6B. The earliest day at which parting of the palpebral fissure began was P13, and that was in the flight group. Completion of disjunction had occurred by P17 in all rats. By Probit analysis, significant differences were found for the ED_{50} for vivarium B versus either flight animals or synchronous controls (Table 4).

Progression of Locomotion

Portions of hind paw print records obtained during walking that are typical of those from which measurements were made are shown in Figure 1. The prints are from rats of different gender and in different groups as well as being at different ages. They show stride increased with age far more than did spread. That was true regardless of the sex of the rat or group to which it belonged.

The progressions for step length and gait width are graphed for the flight and for each of the control groups in Figure 4. Characteristic patterns of change were discernible during development in both stride and spread, though the changes in each measure did not occur at the same time. For example, beginning shortly after P10 there was a steep, steady increase in step length which changed to a plateau period at P30. That, in turn, was succeeded by two further increases in stride beginning at P42 and P51, with each of the latter two phases having successively slower rates (Figure 4A). In contrast to this, gait width precipitously decreased (P14–18) and then increased at a steep rate (P18–26) which, in turn, was followed by a plateau phase (P26–34) and then by an overall gradual increase (P34–81) marked by episodic fluctuations (Figure 4B). Statistical analyses were done on

TABLE 5
Reproductive Trials of Offspring

Group Mates[a]	*Days between Initial Pairing and Birth*[b]	*Number of Live Pups*[b] *female*	*male*	*total*	*Number of Dead Pups*[b,c]
Flight x Flight	24	5.5	6	11.5	1.5
Synchronous x Synchronous	30.5	6.5	6.5	13	0
Vivarium A x Vivarium A	23	6	8	14	0

Notes: [a] individual matings were done with P81 rats (2 male and 2 female) from each group—all pairings resulted in litters; [b] average values for the two matings of each group; [c] pups were counted as dead if they were stillborn and/or cannibalized.

each time segment for stride and spread. There were no significant differences among groups at any interval of time for either measure of walking. During two time periods (P10–30 and P42–51) there were significant differences in step length between genders in all groups, with females having a greater average stride than males (data not shown). Gait spread showed a similar sex difference for a single time interval (P26–81) in which males had a more widely based gait than females. The only instance of a significant "group by time by gender" interaction involving the flight animals occurred with respect to step length at P51–81. In that epoch, stride values for flight males (but not females) decreased, whereas that for males (and females) in each of the control groups increased.

Grooming and Play Behavior

Litters of flight and all control groups were observed for behavioral displays having a prominent somatic motor component, though no systematic attempt was made to quantify these data. Typical rodent grooming habits were observed in members of all groups. Examples include washing of the face with forepaws, licking the body, and scratching with a hind limb.

By P20, rats in all groups exhibited social (e.g., "fighting," wrestling with or chasing of littermates) and locomotor (e.g., bounding, jumping, solo running, and climbing on or clinging to cage top) play.

Mating and Reproduction

An arbitrarily chosen sample of sexually mature (P81) male and female pairs from flight and two of the control groups were placed as a pair in the same cage, both members of a pair being from the same group (Table 5). None of the rats selected for mating came from litters that had experienced perinatal death when they themselves were born, and none were siblings. After two weeks the male was separated from the female in each breeding cage. All females delivered litters. Death was present at a low level among offspring from flight pairs, while it was absent in offspring of control pairs (Table 5).

Discussion

This was one of ten individual but integrated projects of the NIH.R1 payload, which began a series of experiments to explore whether environmental conditions in space—notably microgravity and elevated radiation—affect mammalian development. We ob-

served statistically significant differences between flight and control dams for movement within a novel environment shortly after return to Earth and for birth weight of their pups. There was also higher perinatal mortality among the flight dams' offspring. Conversely among the surviving progeny, *in utero* development under space flight conditions had no effect postnatally on weight gain and attainment of several developmental horizons.

Return to Earth Gravity and Perinatal Events

Shortly after return to Earth there was markedly decreased somatic motor activity by the pregnant rats relative to controls as reported here and by others (Alberts et al., 1995). Postflight motor activity was lowered for a few days in adult male rats recovered from Cosmos biosatellites 605, 782 and 1129 (Serova, 1980), whereas pregnant female rats on Cosmos 1514 were "... active and mobile" upon return to Earth (Serova et al., 1993b). Adult medaka (killifish, *Oryzias latipes*) exhibited a paucity of movement after the fifteen-day IML-2 (STS-65) mission; those fish took about three days to begin swimming normally (Ijiri, 1995). Interestingly, hypokinesia upon return to earth was not evident in the medaka that were fertilized and hatched during their space flight (Ijiri, 1995). We are unaware of any data available to test that idea directly for mammals since none have been born during a space flight. Based on the limited evidence available, this phenomenon of decreased movement after space flight appears to be transient, of wide phyletic occurrence among vertebrates, and only evident in animals that previously experienced Earth gravity.

Perinatal well-being of the offspring was a second area in the NIH.R1 study in which a notable difference was observed between flight versus control groups of rats. The number of pups that were stillborn and/or cannibalized within 12 hours after parturition was greater in the flight group than for the controls. Our values are for deaths that occurred perinatally and before pups were either sacrificed or cross-fostered for separate studies; specifically not included in that count are the two pups, one in each of the vivarium control groups, that died after cross-fostering (see Methods). It is not clear whether the higher mortality among the flight litters was due to a failure to thrive among the offspring, problems with the dam, or both. The percentages we noted have the same trend as was reported for the Cosmos 1514 flight, which covered a longer postnatal time (i.e., 1 week) and involved no cross-fostering. In that case there was 19 percent mortality for the flight group (n=5 dams) and 2.5 percent and 0 percent for the synchronous (n=5 dams) and vivarium (n=5 dams) controls, respectively (Alberts et al., 1986; Serova et al., 1993a). Correlated with the higher mortality of flight offspring is the observation that flight dams had, on average, twice the number of labor contractions of the synchronous controls (Alberts et al., 1995). This finding was confirmed after the NIH.R2 mission (STS-70) (Alberts et al., 1996), and it had been initially noticed for some of the flight dams on Cosmos 1514 (Serova et al., 1993c). No deaths were reported for offspring of the medaka that hatched in space or for those of the F1 generation that hatched on Earth (Ijiri, 1995). Therefore, the phenomenon of diminished survival among progeny may be peculiar to viviparous vertebrates.

Our data on birth weight among the offspring is relevant with regard to the issue of survivability. For example, at normal term gestation in humans, lower birth weight correlates inversely with perinatal mortality (Annis, 1978; Gabbe et al., 1991). All pups weighed within the range of normal for the Sprague-Dawley strain (Donaldson, 1915). However, the pooled values (Table 3) show a steady decline in the average birth weight, which ranked: vivarium controls > synchronous control > flight. Mean weight at birth and the subsequent day for the Cosmos 1514 mission also showed that pups in both the flight

and synchronous control groups weighed less than the vivarium controls (Alberts et al., 1986; Serova et al., 1993c).

Furthermore, in the present study increased morbidity of offspring continued into the F2 generation. Although those rats that were allowed to mate did so and fetuses were carried to term, there was mortality among the (now grown) flight pups' own offspring and none among the controls. Similar results occurred among the F2 generation from the Cosmos 1514 flight (Serova et al., 1984).

Achievement of Postnatal Developmental Milestones

Differences reported here on the events of postnatal development could not be attributed solely to space flight. An example was the growth of the rats. For each group of animals whole body weight plotted as a function of age was a sigmoidal curve, which is typical for mammalian (Zullinger et al., 1984) and other (Brody, 1945; Thompson, 1942) species. The less steep growth curve following weaning that was observed for the flight group relative to controls could be wholly attributed to the peculiar gender ratio in the set of flight rats assigned for this study. Female albino rats normally weigh progressively less than age-matched males as they grow to adulthood (King, 1915; Slonaker, 1912), and there was a preponderance of female rats in the flight group of this study. We have no good way to account for the atypical sex ratio in the flight group assigned for cross-fostering. In a subsequent, independent study (NIH.R2), from P35 to P91 the average body mass of flight offspring (n=8, 5 male, 3 female) was consistently less than that for the synchronous control group (n=9, 4 male, 5 female) though not to a statistically significant degree (Fuller et al., 1996). In each group over that time period, female rats weighed significantly less than males (Hoban-Higgins, personal communication).

Developmental horizons occurred at times that did not differ significantly between flight and synchronous control groups, all of which fell within the range of time described as normal in other studies. For example, rats typically walk before they open their eyes (Altman & Sudarshan, 1975; Bolles & Woods, 1964; Tilney, 1933). The range of time found for eyelid opening in flight and control pups (P13–17) in this study matched that reported for normal albino rats (Addison & How, 1921; Bolles & Woods, 1964; King, 1923; Tilney, 1933), and extends findings reported from the Cosmos 1129 (postflight mating) and 1514 (preflight and postflight mating) experiments (Serova & Denisova, 1982; Serova et al., 1993a). Eyelid opening is the end result of epithelial changes—which are influenced by epidermal growth factor (Hoath, 1986), and environmental stimuli (Smart et al., 1990), and, probably, neuromuscular events associated with the action of the levator palpebrae superioris muscle and its innervation. Since there was no statistically significant difference between flight and synchronous control pups these mechanisms would seem to be unaffected by the conditions experienced prenatally during space flight. However, it is interesting that there were statistically significant differences between vivarium B controls versus either flight or synchronous controls, and the ranking for the groups was the same as that for birth weight.

The sensorimotor integration required for the initiation of walking developed over a normal time course (P4–13) in the flight (and control) rats of this study relative to values previously reported for Earth-bound rats (Altman & Sudarshan, 1975; Bolles & Woods, 1964; Tilney, 1933; Westerga & Gramsbergen, 1990). Once begun, walking progressed in very similar fashion in each group of rats when hind paw placement was used as the end point. For the hind limbs, stride increased at a much faster rate than did spread. Although

the overall trend was an increase in both hind limb step length and gait width, several distinct phases for each of those measures appeared as the rats grew, and the epochs differed in their time of occurrence when comparing the two measures. The finding that the phases of walking for flight rats were in synchrony with those of the control animals suggests that the integrated neuromuscular substrate for locomotion matured on a schedule considered to be normal.

For certain developmental time periods, there were statistically significant differences in hind limb use when comparing between genders irrespective of group, suggesting that sexual dimorphism was operant more than any effect due to space flight. For example, during the time epoch from approximately week four onward, the least squares means (LSM) for male hind limb spread during walking was significantly more than that of females (data not shown). Sex differences relating to stride in our study showed male rats as having significantly smaller LSM values than females during two of the four time epochs studied. These observations are consistent with other studies (Mullenix et al., 1975; Parker & Clarke, 1990) of gender-related differences in adult rats in which males had a wider gait but not necessarily a longer stride than females. In summary, hind paw placement during locomotion in growing rats differed more between sexes than when comparing those exposed to microgravity during gestation with any of the control groups.

Our observations on hind limb use during locomotion are for neonates that developed prenatally in microgravity and initiated walking several days after return to a 1g environment. They contrast with observations on rats that underwent an extended microgravity experience postnatally and whose locomotion was tested shortly after return to Earth. Measurement of hindquarters elevation and leg extension in young adult rats after a fourteen-day space flight (STS-58) revealed peculiarities that produced "atypical foot placement" for flight rats relative to controls; differences abated within a week (Fox et al., 1994). Preliminary kinematic data after a nine-day space flight (NIH.R3, STS-72) showed that rats exposed to microgravity during their second postnatal week exhibit prolonged extension at the ankle during the stance phase of locomotion when tested at recovery (Walton et al., 1996).

Other complex activities requiring sensorimotor integration were achieved by flight rats in the present study. Social and locomotor play—behaviors typically performed by young birds and mammals (Bekoff & Byers, 1981; Fagen, 1981)—were evident in rats of the flight and control groups at the age they normally occur in albino pups (Bolles & Woods, 1964; Tilney, 1933). At adulthood, flight pups completed coitus which resulted in the birth of mostly viable offspring.

Analysis of Selected Findings

Two postnatal measures—birth weight and eye opening—showed values for the flight pups were significantly less than those for vivarium B control group. Perhaps more importantly, there was a consistent gradation from one of those extremes to the other (see Tables 3 and 4). That suggests there may be an encompassing variable acting additively in the groups, being least in the vivarium B controls and most in the flight group. An obvious candidate is stress. The experimental design included the stresses of transiently increased auditory and gravitational stimuli (launch and landing), confined living space (AEMs) and surgery (hysterectomy and laparotomy). They were present to different extents among the animal groups. Flight rats had the broadest exposure—all of them; the vivarium B rats had none of them. The other two control groups experienced these stressors to different inter-

mediate degrees in that continuum (Table 1). Various types of stress experienced by rat dams during pregnancy have been shown to affect their progeny adversely (see, for example, Naumenko, 1984; Moberg, 1985).

A separate consideration is that there were virtually no statistically significant differences postnatally when comparing between only the flight and synchronous control groups, and there was greater perinatal morbidity in the flight group. Could that lack of differences be reflecting the demise of less fit flight animals that left only those most like the synchronous controls? We do not think that is a likely explanation. Only one of the four flight pups that died perinatally would have been assigned to this study. (The one synchronous control pup that died perinatally would not have been assigned for this study based on the predetermined allotment regimen.) Thus, only one more pup would have been included in the flight group. It is doubtful that a single additional inclusion would have resulted in a statistically significant difference between flight and synchronous control groups for all measures we compared, though we cannot discount that possibility for isolated measures (e.g., initiation of walking).

Summary

Our results, when viewed in the context of other studies, lead to the following conclusions. Prenatal space flight, per se, neither precludes, hastens nor delays attainment of end points that result from mechanisms which control postnatal weight gain, eyelid opening, and walking. Where significant differences between groups did occur in postnatal measures we took, they were generally between the flight and vivarium B control groups. Such differences are better attributed to a variable(s) not peculiar to space flight, for example stress. Second, return to Earth after space flight is accompanied—in the short term and for the adult, in this case the pregnant rat—by clearly decreased movement. Finally, although in most cases parturition is normal, space flight for the pregnant rat is more likely to coincide with perinatal death of its progeny. This, too, may be stress related.

Acknowledgments

We thank L. Dubrovin, C. Elland and D. Leonard for unstinting assistance before and at the time of the mission; K. Williams for attention given to the foster litters upon their arrival at the University of Idaho; J. Alberts, A. Ronca and D. Reiss-Bubenheim for providing postflight videotapes of the dams; T. Cimino, K. Parkman, S. Weinberger and J. Zinck for their care and dedication while helping to gather data; D. Everson for help on statistical analysis; and J. Alberts, S. Anderson and two anonymous reviewers for constructive comments on drafts of this manuscript. The study was supported by NASA-Ames: Cooperative Agreement No. NCC2–862.

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