# Motor System Development Depends on Experience: A microgravity study of rats

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## ABSTRACT

Animals move about their environment by sensing their surroundings and making adjustments according to need. All animals take the force of gravity into account when the brain and spinal cord undertake the planning and execution of movements. To what extent must animals learn to factor in the force of gravity when making neural calculations about movement? Are animals born knowing how to respond to gravity, or must the young nervous system learn to enter gravity into the equation? To study this issue, young rats were reared in two different gravitational environments (the one-G of Earth and the microgravity of low Earth orbit) that necessitated two different types of motor operations (movements) for optimal behavior. We inquired whether those portions of the young nervous system involved in movement, the motor system, can adapt to different gravitational levels and, if so, the cellular basis for this phenomenon.

We studied two groups of rats that had been raised for 16 days in microgravity (eight or 14 days old at launch) and compared their walking and righting (ability to go from upside down to upright) and brain structure to those of control rats that developed on Earth. Flight rats were easily distinguished from the age-matched ground control rats in terms of both motor function and central nervous system structure. Mature surface righting predominated in control rats on the day of landing (R+0), while immature righting predominated in the flight rats on landing day and 30 days after landing. Some of these changes appear to be permanent.

Several conclusions can be drawn from these studies: (1) Many aspects of motor behavior are preprogrammed into the young nervous system. In addition, several aspects of motor behavior are acquired as a function of the interaction of the developing organism and the rearing environment; (2) Widespread neuroanatomical differences between one-G- and microgravity-reared rats indicate that there is a structural basis for the adaptation to the rearing environment. These observations provide support for the idea that an animal's motor system adapts for optimal function within the environment experienced during a critical period in early postnatal life.

# INTRODUCTION

Professional baseball players can hit a small, round object traveling over 100 miles an hour with a stick. If their timing is just right and they put the full force of their bodies into the swing, they can hit this object (a ball) over 500 feet through the air. Hitting a speeding ball requires that batters match the trajectories of the ball and the bat perfectly. That is, they must predict when the ball and bat will occupy the same space. Even the smallest error means that they will miss the ball completely.

To hit a home run, a batter must predict the effect of gravity on both the ball and the bat—and have exquisite motor control. Not only adults have such abilities. A professional baseball player probably first hit a home run at age eight or nine years. This skill was developed during youth as the player learned to control and coordinate movements in Earth's gravitational field. We are interested in understanding the processes underlying the development of such controlled movement to determine whether there is demonstrable "tuning" of the central nervous system (CNS) to the Earth's gravitational field during development.

Movement control begins for baseball players, as for all people, soon after birth. At birth, a baby can kick its arms and legs and lift its head. Within months it can roll over, sit up, and begin to crawl. Between its first and second birthdays, a toddler will take its first steps. Running and jumping are not far behind. During all this time, planning and controlling movements must include calculations for the effects of gravity on the body as well as on the world. Thus, it seems that gravity is as essential to the development of motor function as light is to the development of vision.

We expected that if gravity were reduced or removed, motor system development would be profoundly altered. Indeed, the microgravity of spaceflight represents a fundamental alteration in an animal's environment. The constellations of sensory signals stimulated by gravity, such as righting reactions (turning upright when placed upside down), are not elicited. The perceptual realm has been modified, but motor experience is also altered. In the presence of gravity, animals must negotiate a two-dimensional space. In microgravity, animals must develop a strategy to negotiate a three-dimensional space. The sensory-motor transformation in these animals must change. Further, the internal representation of reality built as the animal explores and interacts with its environment must differ from the internal representations of animals that are reared on the ground. This view is supported by the results of our ground-based studies as well as by studies of the postnatal development of sensory systems. Yet, our view can be tested directly only if young animals are studied after they spend a period of development in microgravity.

It is well known that vision and hearing, for example, do not develop normally if animals are deprived of normal input for those sensory systems. The reduction or absence of stimuli must occur, however, during a particular period of development—one during which the nervous system is particularly sensitive to environmental stimuli. Hubel and Weisel, who identified such a period in their studies of the cat visual system, named this time a "critical period of development" (Hubel,

1970). Over the last 30 years, such periods of development have been identified for many sensory systems (Katz, 1996). There is even a critical period for the acquisition of primary songs in songbirds and of language in humans (Doupe, 1999).

Anatomical correlates have been identified for behaviorally defined critical periods of development. Indeed, as a young animal interacts with its environment, the patterns of activity in the animal's CNS profoundly influence CNS structure. This has been shown most extensively for the visual system (Katz, 1996). Work from our lab also supports the view that the development of motor skills is fundamentally linked to experience. If the hindlimbs do not support an animal's weight during the first weeks of its life, coordinated movements such as swimming and walking are profoundly altered (Walton, 1992). Modifications in electrical or chemical signals reaching the spinal cord during this same period of development lead to changes in the structure of the CNS (i.e., to alterations in the motor neuron dendritic architecture and the expression of surface molecules) (Kalb, 1994).

The Neurolab mission provided an opportunity to test our hypothesis that postnatal development incorporates an experience-dependent adaptation of the CNS to Earth's gravity. Thus, the nervous system of animals that spend a critical period of development in space would be adapted to the microgravity environment. When these animals returned to Earth, this adaptation would be evident in the way the animals moved and in the structure of those regions of their nervous system that support motor function.

# METHODS

Our investigations aimed to study systematically the effect of microgravity exposure in early life on the motor system using behavioral, anatomical, and molecular studies.

### Animals

Three groups of rats were studied; flight, ground control, and basal control. These were divided again into two groups according to age. The younger rats were launched on postnatal day 8 (P8) and landed on P24. The older rats were P14 at launch and P30 at landing. The flight rats spent the intervening 16 days in low Earth orbit. The ground control rats remained at the Kennedy Space Center (KSC). Half of the control rats were housed in standard shoebox-sized (vivarium) cages. The other half of the control rats were housed in smaller cages that were matched to those of the flight rats (asynchronous ground control (AGC)). The basal groups were studied when they were P8 or P14; that is, they were age-matched to the day of launch.

### Behavioral studies

For the behavioral studies, the older flight and AGC rats were videotaped during the mission on flight day 6 (FD6). The younger rats were videotaped on FD15. The rats were studied in a general-purpose workstation (GPWS) in the Spacelab module (spaceflight) or at KSC (AGC). They were videotaped at

60 frames per second (fps) while they moved about an "animal walking apparatus." This comprised a platform that was covered with foam on one side and a grid on the other.

After landing, three behaviors were used to evaluate the effect of microgravity on motor system function: swimming, free walking, and surface righting. The flight and control rats were videotaped at 60 fps during swimming, and at 200 fps during walking and surface righting. In all cases, the hindlimb joints were marked with a felt pen. For swimming, each rat was carefully placed near one end of a tank of warm water. When the rat reached the other end of the tank, it could leave the water. The rat was then picked up and placed in the water for another trial. Each session lasted two minutes and comprised up to 15 trials. Walking was tested following a similar paradigm using a walking stage. Walking sessions could last up to 10 minutes per rat. Each surface righting trial began by holding the rat on its back with its head straight. Once the animal had relaxed, it was released from this position. Direct frame-by-frame analysis or computer-assisted motion analysis (Motus 5, Peak Performance, Inc.) was used. Statistical analysis used Statview software (SAS Institute, Inc.).

## Anatomical studies

Neurons in the cervical spinal cord, neocortex (hindlimb area), and supraoptic nucleus of the hypothalamus were studied.

The spinal cord studies focused on the younger group of rats. The spinal cords were collected on FD7 and FD14 and a few hours after landing on recovery day zero (R+0). They were preserved by perfusion fixation. Motor neurons were labeled retrogradely with a dye (DiI) applied to the ventral roots of the cervical spinal cord. Tracings of neurons and measurements of neuronal characteristics were made using computer-assisted camera lucida software (Neurolucida) (Inglis, 2000).

Our studies of the brain regions focused on the older rats. Brain tissue collected on landing day and 18 weeks after landing was fixed by immersion. Sections were made through the cerebral cortex or hypothalamus. In the case of the cortical tissue, once the cortical layers were identified, serial ultra-thin sections were cut, stained with uranyl acetate and lead citrate, and examined in a Jeol-1200 EX electron microscope. Synaptic density per unit area was estimated from 10 electron microscope samples of neuropil from each layer (layers I, II/III, IV, Va, Vb, and VI) from each rat. The cross-sectional lengths of synaptic junctions (synaptic apposition length) of all synaptic profiles were measured in the prints using a magnetic tablet (SummaSketch III) and the Scion Image image analysis program (Scion Corporation, Frederick, MD) (DeFelipe, 2002).

The right and left half halves of the hypothalamus were processed separately. A small block containing the supraoptic nucleus was dissected from the one block and processed for electronmicroscopy. Serial sections were cut from the other block using a Vibratome. These were processed for oxytocin and vasopressin immunohistochemistry (García-Ovejero, 2001). In all our studies, differences with $p < 0.05$ were considered to be significant.

## RESULTS

Behavioral and anatomical studies found clear differences between both ages of flight and control rats on the day of landing. Some of these differences were transient, while other differences persisted for as long as the rats were studied. There were no consistent differences, however, between the two groups of control rats.

### Growth

We first examined the effect of microgravity and cage design on the growth of the rats. Figure 1 is a plot of body weight as a function of days after landing for flight (●), AGC (○), and vivarium (◆) rats. The younger flight rats (Figure 1A) were significantly smaller than the cage-matched AGC rats from the day of landing (R+0) through R+7. Flight animals were significantly smaller than the vivarium control rats through R+12. The cage itself influenced the growth of these rats. The control rats housed in flight-like cages (AGC rats) were significantly smaller than vivarium rats R+0 through R+5. Spaceflight did not influence the growth of the older rats (Figure 1B). In fact, there was no significant difference between flight and AGC rats from R+0 through R+30. Again, the cage itself did influence their growth. The AGC control rats were significantly smaller than vivarium rats between R+1 and R+9. The vivarium rats were significantly larger than flight rats between R+5 and R+8.

### Behavior

Clear differences between flight and control rats were observed in all of our behavioral tests. Similar changes were seen in the younger and older groups of rats. This report will focus on our findings in the older rats. Further, although the older AGC rats were lighter than their age-matched vivarium control rats, this did not influence the behavioral parameters that we studied.

The most marked difference between flight and control rats was the combination of movements they used to right themselves. When a control rat is placed on its back and released, it rights itself in a controlled sequence as shown in Figure 2. First, it rotates its head, then the forelimbs, and then the hindlimbs. This tactic, called "axial righting," is typical of adult animals. Young animals do not always use this tactic (Pellis, 1991). For example, when young animals are released, they may rotate their head and forelimbs in one direction and their hindlimbs in the opposite direction. This is called "corkscrew righting." Another major righting tactic that we saw we called "ventroflexion." In this case, the animal lifts the front and back legs toward each other so that the body forms a U-shape. The front of the animal then falls in one direction and the hindlimbs follow.

Before launch, the rats used all three tactics to right themselves. This is shown in the first column of Figure 3 where the three tactics are color-coded. Trials using axial righting are blue, corkscrew righting trials are green, and trials incorporating
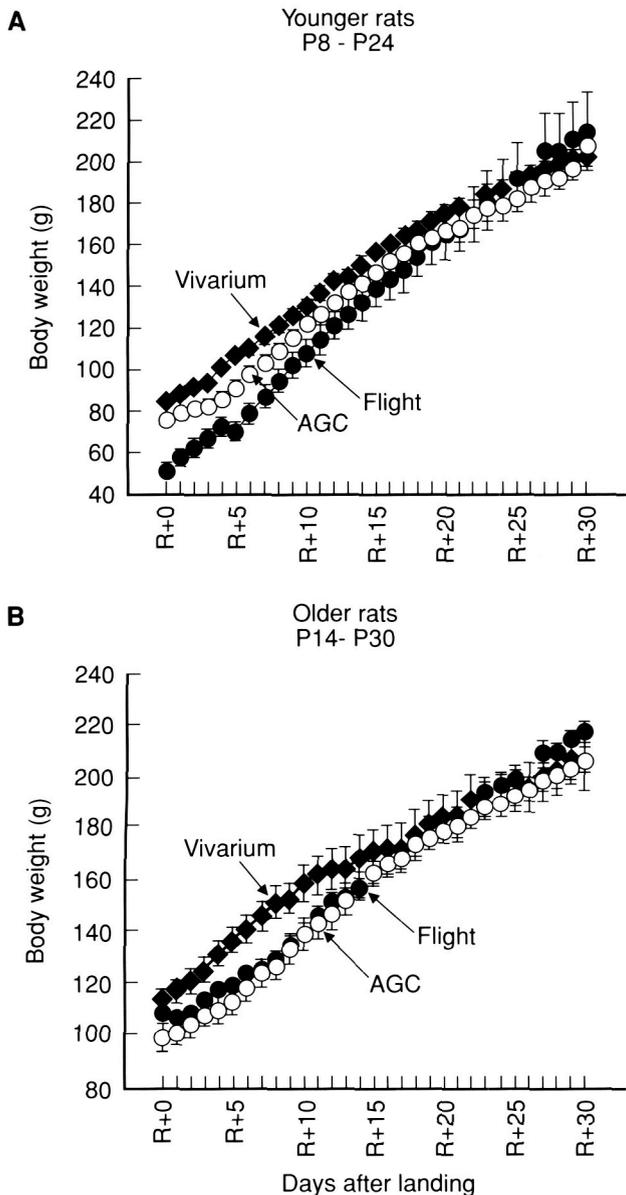
**A**

Younger rats
P8 - P24



**B**

Older rats
P14- P30



Days after landing

**Figure 1.** Plot of body weight as a function of days after landing in flight (●), AGC (○), and vivarium (◆) rats. (A) Younger animals. Flight rats were lighter than cage-matched control rats until R+8 and lighter than vivarium controls until R+13. Cage effects were also seen. AGC animals were lighter than vivarium control rats until R+6. (B) Older animals. Spaceflight did not affect the weight of this group, but there was a cage effect.

ventroflexion are red. Axial or corkscrew tactics were used in over 80% of the trials on P14. Sixteen days later, when the rats were P30, axial righting predominated in the control rats. This is shown in the second column. The same acquisition of mature righting tactics did not occur in the flight rats. In fact, as shown in the third column in the figure, axial and corkscrew righting tactics were used in fewer cases than at launch. Ventroflexion, the most immature tactic, predominated. Furthermore, mature righting tactics did not predominate in the flight rats even 30 days after landing.

Flight rats were able to walk and swim when they were tested a few hours after landing. Only walking will be discussed here. When the flight rats were placed on the stage on R+0, they walked freely and explored their immediate surroundings. At first glance, they seemed to be quite similar to the control animals. On careful inspection, however, it appeared that the flight rats stayed close to the ground and seemed to tilt their hindquarters from side to side as they walked. To examine this more closely, the position of the hindlimb was entered into a computer using markings on the skin of the animal.

Seven points represented the hindlimb: tip of the toes, metatarsophalangeal joint, ankle, knee, head of the femur, ischial tuberosity, and iliac crest. Line segments connected these points to generate a stick figure of the leg as shown in Figure 4A. The stick figures show five positions of the right leg as a flight rat takes one step from left to right. First the rat puts its foot on the ground (foot contact (FC)), This marks the beginning of what is called the stance phase of the step cycle. Next, the knee bends as the rat places weight on the leg. Third, the rat extends the right leg and shifts its weight to the left leg (not shown). This is in preparation for foot lift (FL). Foot lift marks the end of the stance phase and the beginning of the swing phase. Once the foot is off the ground, the rat swings the leg forward. Finally, the foot is placed on the ground to begin another step.

We measured the distance between the head of the femur and the walking surface in the flight and control rats on R+0. Results are shown in Figure 4B for one flight (red) and one control (blue) rat. The distance is plotted as a function of time for two steps. The phases of the step cycle are marked by the broken lines. During the stance phase, the flight rat brings its hip closer to the ground than the control rat does. This leads to a large hip excursion in the flight rat (double arrow, Figure 4B) compared to the control rat. This finding is consistent with our initial observation that the flight rats seemed to stay closer to the ground and to tilt from side to side more than the control animals. We calculated the hip excursion in all the flight and AGC control rats on R+0. We found that the mean excursion was significantly larger in flight rats than in control animals on (p<0.05, unpaired t-test). This is shown in Figure 4C.

**Anatomy**

Our morphological studies demonstrate that structures in the CNS of flight rats differed from those of rats reared on Earth.

In the first study, we examined the dendritic architecture of cervical motor neurons using the labeling dye DiI (Figure 5). There were no differences between the dendritic trees of flight and control motor neurons when the tissue was obtained before landing (on FD7 or FD14). In contrast, the dendritic trees of some motor neurons from flight rats sacrificed on R+0 were significantly smaller and less branched than the dendritic trees of age- and cage-matched control rats (Inglis, 2000).

We sought to determine why some motor neurons in flight animals were affected by microgravity while other motor neurons were not. Toward this end, we divided the motor neurons
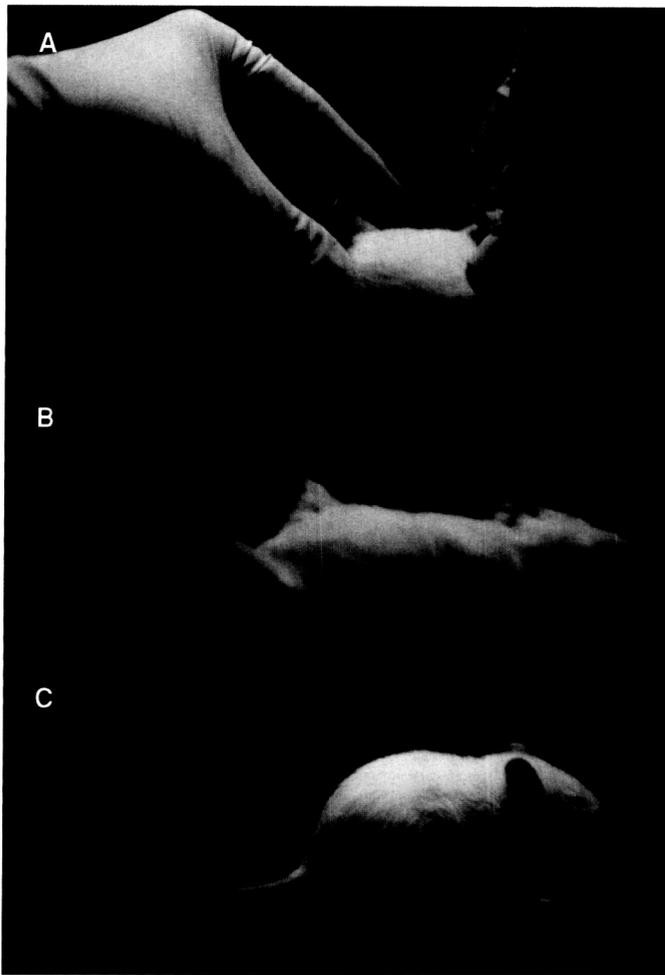
**Figure 2.** Axial righting in a control rat. (A) The rat is held upside down. (B) When it is released, first the head, then the forelimbs, and finally the hindlimbs rotate in the same direction. (C) This achieves the right-side-down posture shown here.
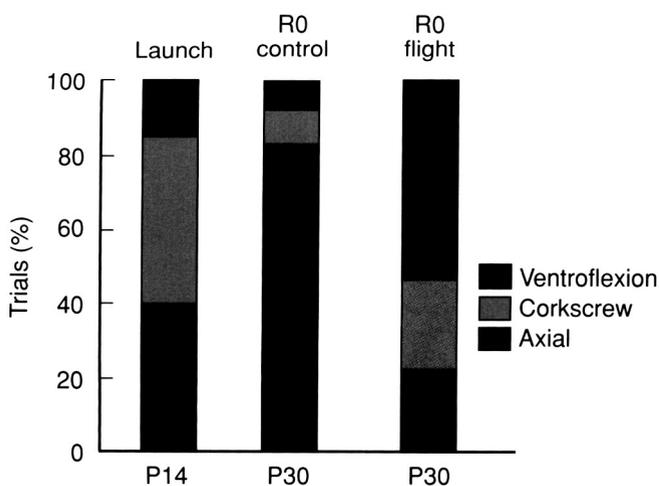


**Figure 3.** Tactics used in surface righting before launch and on the day of landing. Rats right themselves using: ventroflexion (red), corkscrew (green), or axial (blue) tactics. Mature (axial) righting tactics predominate on R+0 in control rats but not in flight rats.
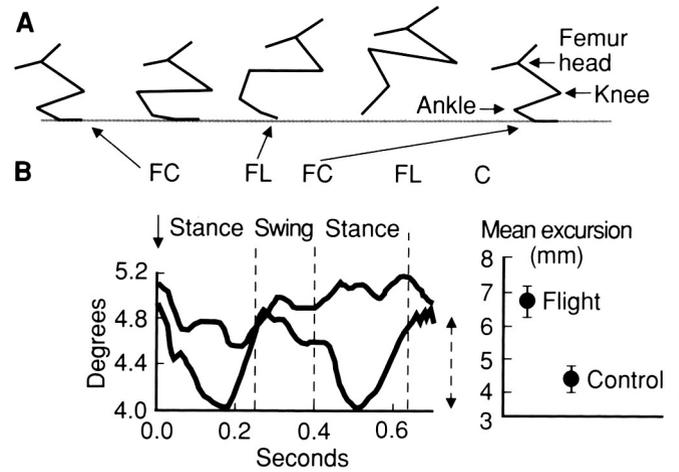


**Figure 4.** Walking on R+0. (A) Stick figure showing the position of the hindlimb of a rat moving from left to right. (B) Plot of distance of the hip from the ground during two steps for a flight rat (red) and a control (blue) rat. (C) Excursion of the hip (broken vertical line) in flight rats and control rats on R+0 (mean±s.e.m.) showing significantly greater movement in the flight rats. (FC, foot contact; FL, foot lift)
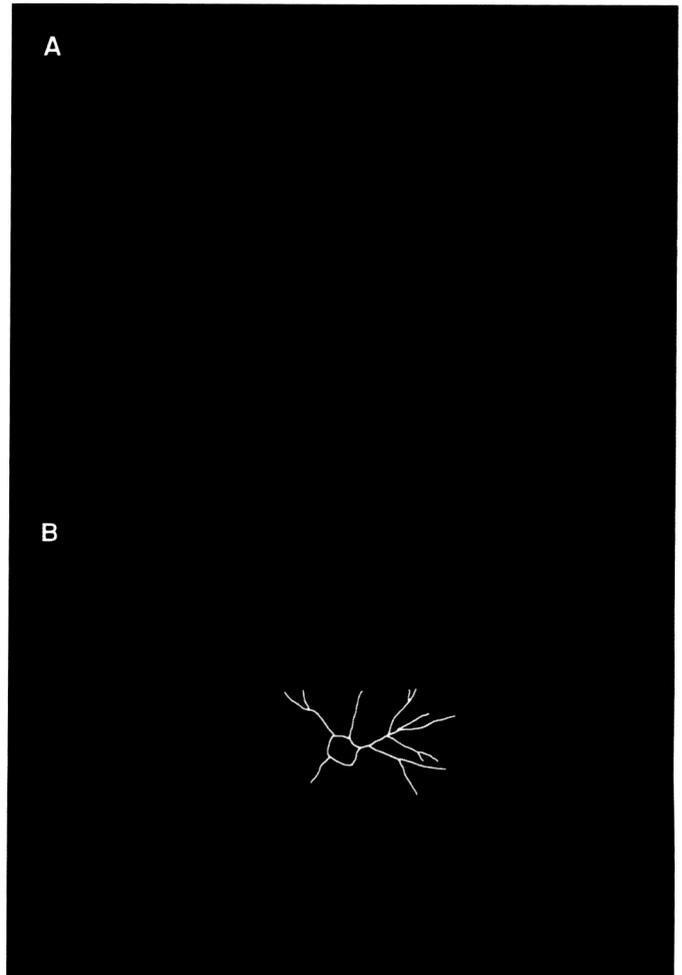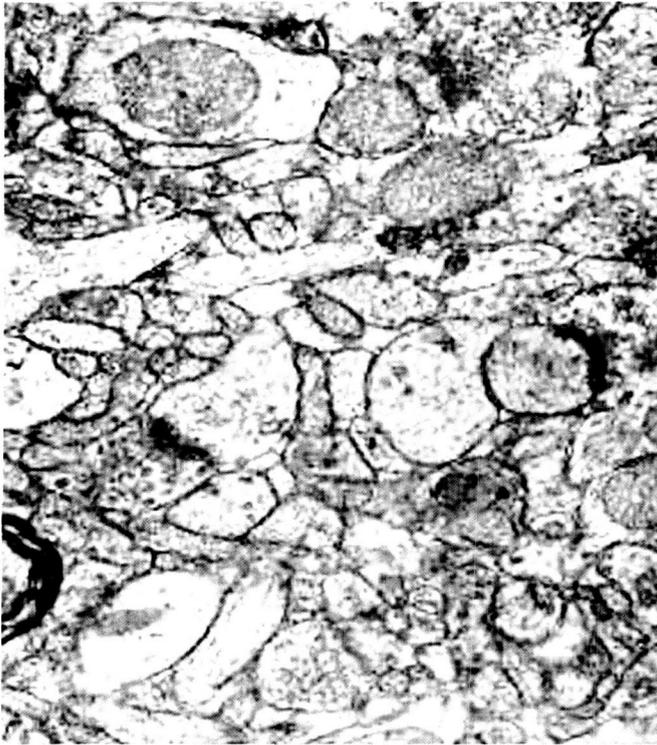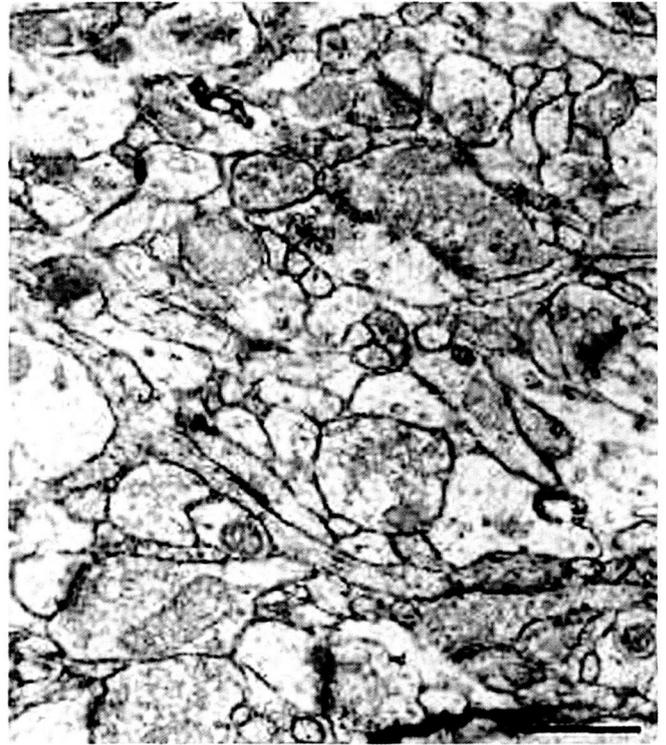


**Figure 5.** Motor neurons in the cervical spinal cord labeled with the dye DiI. (A) Low-power micrograph showing location of motor neurons in the ventral horn. (B) High-power micrograph of one DiI-filled motor neuron. Superimposed white lines indicate location of the soma and some dendritic branches.

**Control**          **Flight**

**Figure 6.** High-power electron micrographs illustrating the neuropil (interwoven nerve cell processes) of layer II/III from a control and a flight rat. Scale bar: 0.5 microns.

into subgroups based on their location within the ventral horn of the spinal cord. We performed this analysis because it is known that the topography of motor neuron cell bodies reflects the pattern of muscle innervation. When subjected to this analysis, we found that motor neurons located in the medial portions of the ventral horn were very profoundly influenced by rearing in microgravity, while laterally located motor neurons were identical in flight rats and control animals. Medially located motor neurons innervate the muscles involved in postural control and surface righting, so this result is consistent with our behavioral findings.

Differences between flight rats and control rats were also seen in the region of cerebral cortex that is related to hindlimb function. This investigation focused on the older rats and examined the characteristics of individual synapses. The quality of the images, as seen in the electron microscope, was excellent (Figure 6). It was thus possible to measure accurately the cross-sectional length of synaptic contacts between neurons. We were also able to determine whether synapses were symmetrical (densities on both sides of the synaptic cleft) or asymmetrical (density on one side only). Development in microgravity leads to changes in the number and morphology of cortical synapses in a laminar-specific manner. In tissue obtained on R+0, the synaptic cross-sectional lengths were significantly longer in flight rats than they were in ground control rats in layers II/III and layer Va. Differences were only seen in asymmetrical synapses, which are known to be excitatory.

During the 18-week period after landing, synapse cross-sectional length increased in both control rats and flight rats. This was statistically significant in all layers in both groups of rats with one exception. There was no change in layer IV synapses in flight rats.

The increase was greater in control rats than in flight rats. This unequal growth had two consequences: (1) Synapse cross-sectional length in control rats increased to reach that seen in flight rats on R+0. Thus, the significant differences in layers II/III and Va seen on R+0 were abolished. (2) Synaptic cross-section length in layer I was significantly greater in control rats than in flight rats by R+18 weeks.

Finally, we examined neurosecretory neurons in the supraoptic nucleus of the hypothalamus at R+0 and R+18 weeks. This study focused on the large cells that secrete the hormones oxytocin or vasopressin. Several signs of enhanced transcriptional and biosynthetic activity were observed in magnocellular supraoptic neurons of flight rats on R+0 compared to control rats. These include increased c-fos expression, larger nucleoli and cytoplasm, and higher volume occupied in the neuronal perikaryon by mitochondria, endoplasmic reticulum, Golgi apparatus, lysosomes, and cytoplasmic inclusions known as nematosomes. In contrast, the volume occupied by neurosecretory vesicles in the supraoptic neuronal perikarya was significantly decreased in flight rats. This decrease was associated with a significant decrease in oxytocin and vasopressin immunoreactive levels, suggestive of an increased hormonal release.

Vasopressin levels, cytoplasmic volume, and *c-fos* expression returned to control levels 18 weeks after landing. However, oxytocin levels were still reduced in flight rats compared to control rats.

## DISCUSSION

Our results demonstrate that: (a) motor system development clearly depends on the interaction of the animal with its environment, (b) the CNS is sensitive to changes in the gravitational field, and (c) there is a critical period for motor system development.

Development can be considered as incorporating four processes: growth, maturity, adaptation, and learning. Our results concern all four processes.

### Growth

Microgravity itself did not affect body growth. This is seen in the older rats where there were no significant differences in the body weight of AGC rats and flight rats (Figure 1B). The low weight of the younger flight animals on R+0 was a secondary result of the microgravity environment. It seemed to be due to a reduced interaction between the mother and younger rats in microgravity. This was critical for the younger rats since their eyes were still closed at launch and for the first days of the mission. Nevertheless, this did not have long-lasting effects since the flight rats had reached the weights of the AGC rats by a week after landing (Figure 1A). The nature of the cages did influence body growth, however. This is evidenced by the greater weight of the vivarium rats compared to the AGC control rats in both age groups (Figure 1). Nevertheless, in the behavioral tests, no differences were seen between vivarium rats and AGC control rats. One anomaly is worthy of comment. The older vivarium rats were significantly larger than the flight rats between R+5 and R+8. As indicted by the shape of the graph (Figure 1B), this was probably due to weight gain by the vivarium rats rather than to a five-day delayed influence of spaceflight on the flight rats.

### Surface righting

Maturation is the acquisition of adult characteristics. We have shown that the acquisition of adult movement tactics, such as how to turn over, is not hardwired into the CNS. Rather, it is an example of an adaptation to the environment and requires specific goal-directed activity. When placed on its back in a gravitational field (Figure 2A), a rat will do whatever is needed to turn over. This can be accomplished in a number of ways as seen in young rats (first column, Figure 3). As rats grow older, the most efficient tactic, axial righting, comes to predominate (second column, Figure 3). This maturation does not occur in flight rats (third column, Figure 3), however, because righting is not an appropriate movement in microgravity. In fact, it does not even occur in microgravity. When flight rats are released from a supine position, they float up without rotating their bodies. This is not surprising since there is no vestibular input to signal "up" and "down." These results indicate that

mature surface righting tactics arise as an adaptation to gravity and require performance to acquire.

It is worth noting that the flight rats were able to right themselves the first time they were tested on R+0. That is, the signals from the peripheral vestibular apparatus were sufficient to signal that the rat was upside down. This indicates that the critical period for the establishment of reflexes between the inner ear and the neck (vestibulo-collic reflexes) ends before P14.

Furthermore, even 30 days after their return to Earth, the flight rats had not acquired mature righting characteristics. That is, there is a critical period for the development of mature surface righting tactics. This period ends on or before P30.

### Walking

When they launched, the older rats had their eyes open and could walk and run on a flat surface. However, mature locomotion was not yet achieved. Thus, any changes in locomotion during the mission would reflect continued maturation of the motor system and adaptation to the environment. The flight rats remained close to the ground and tilted from side to side when they walked on R+0. That downward tilt was due to a flexion of the leg during the stance phase (Figure 4). This suggests that the supporting leg flexed when weight was shifted from the other leg. It seemed that the rat was not prepared to support its weight on its hindlimbs. This is consistent with locomotion in a microgravity environment where the hindlimbs do not support weight. In fact, as in astronauts, locomotion in animals is accomplished largely by the forelimbs (or arms).

On Earth, limb extension is used when the animal pushes against the ground at the end of the stance phase. In fact, extensors are sometimes called "antigravity" muscles because they exert force against gravity. This does not occur in microgravity. If there is reduced joint extension as an animal walks, it will not move away from the surface. Rather, it will appear to be walk close to the ground as seen in the flight rats. This type of walking reflects an adaptation of the flight rats to the microgravity environment.

### Anatomy

The structural changes in the brain and spinal cord demonstrate that neurons subserving motor functions undergo activity-dependent maturation in early postnatal life in a manner that is analogous to sensory systems such as vision or hearing. These changes are consistent with, and provide a structural basis for, the behavioral results. This part of our study was carried out on the younger rats that did not grow well in microgravity. It is reasonable to ask if the weight differences between flight rats and control rats could account for the difference seen in dendritic structure. We do not think this is the case since differences between flight rats and control animals were only present in specific areas—i.e., in the motor neurons in the medial and not the lateral aspect of the ventral horn.

There are functional consequences of the anatomical changes found in the dendritic tree. A reduction in motor neuron dendritic tree size and complexity will influence the

compartmentalization of electrical and chemical signals received by dendrites. In addition, this reduction is likely to alter the number and types of synaptic inputs these cells receive. The alterations in neuronal information processing will be reflected in the output of the cell. As a consequence of changing the firing pattern of the neuromuscular unit—particularly of postural musculature—animal behavior is affected (Inglis, 2000). For example, a lack of vestibular input in microgravity could alter the development of dendritic architecture. This could, in turn, contribute to changes in motor neuron activity when the input is restored, as when the flight rats return to Earth. These experiments were not designed to provide a direct link between our anatomical and behavioral data. Nonetheless, they do suggest that the rearing environment will influence the operation of the neurons involved in motor function.

Our findings in the cerebral cortex also support this view (DeFelipe, 2002). On R+0, the cross-sectional lengths of excitatory synapses were longer in flight rats than in control rats. This increase may be interpreted in terms of the characteristics of the microgravity environment. Microgravity provides a three-dimensional space for locomotion. Thus, it is "enriched" with respect to the two-dimensionality experienced by rats on the ground. Indeed, flight rats are free to "walk" along all six surfaces of their cages, not just on one surface (the bottom) as on Earth. This may stimulate an increased contact area between neurons as has been found when rats are raised in a cage that contains toys and additional walking surfaces.

After landing, synapses in the flight rats continue to grow, but not as fast as in the control rats. Thus, after 18 weeks, the control rats were able to "catch up." It is possible that the two-dimensional environment of the Earth was not sufficient to stimulate growth of the cortical neurons. The critical period for cortical development had not ended. But, when the flight rats landed, they were deprived of the necessary stimuli (locomotion in three dimensions) needed for development. Only neurons in layer IV did not change significantly after landing. This suggests that critical periods of development may differ among the cortical layers.

The spinal cord and hindlimb area of the cortex were chosen for study because they have a direct role in the organization and control of movement. In contrast, alterations in hypothalamic neurosecretory neurons reflect effects of microgravity on the volume of body fluids outside of cells (which has both a direct and an indirect effect on CNS function) as well as the stress associated with spaceflight. We thought it was important to carry out such a study in the same rats examined in our other studies.

Our results, showing increased activity on R+0, are in agreement with studies in adult rats, suggesting an increased cellular activity in the hypothalamic-pituitary neurosecretory system after spaceflight. These effects are probably associated with osmotic stimuli resulting from modifications in the volume and distribution of body fluids and plasma during spaceflight and landing, and they may reflect normal physiological adaptations (Garcia-Ovejero, 2001). These changes, and the resulting modifications in vasopressin regulation, may be involved in the transient alterations in renal function observed in experimental animals and astronauts after spaceflight. In addition, spaceflight in young animals resulted in permanent modifications in oxytocinergic neurons, probably related to stressful conditions associated with spaceflight and landing. These irreversible modifications may have permanent effects in different functional, behavioral, and neuroendocrine parameters.

The present observations provide strong support for the idea that the motor function of an animal adapts for optimal operation within the environment experienced during a critical period in early postnatal life. Further studies should be designed to explore the possibility of permanent behavioral, neuroanatomical, and endocrine changes associated with exposure to microgravity during critical postnatal developmental periods.

## Acknowledgements

## REFERENCES

THE PERIOD OF SUSCEPTIBILITY TO THE PHYSIOLOGICAL EFFECTS OF UNILATERAL EYE CLOSURE IN KITTENS. D.H. Hubel, T.N. Wiesel. *J. Physiol.* (Lond), Vol. 206, pages 419–436; 1970.

A DESCRIPTIVE ANALYSIS OF THE POSTNATAL DEVELOPMENT OF CONTACT-RIGHTING IN RATS (*rattus norvegicus*). V.C. Pellis, S.M. Pellis, P. Teitelbaum. *Dev. Psychobiol.*, Vol. 24, pages 237–263; 1991.

IDENTIFICATION OF A CRITICAL PERIOD FOR MOTOR DEVELOPMENT IN NEONATAL RATS. K.D. Walton, D. Lieberman, A. Llinas, M. Begin, R.R. Llinas. *Neurosci.*, Vol. 51, pages 763–767; 1992.

ELECTRICAL ACTIVITY IN THE NEUROMUSCULAR UNIT CAN INFLUENCE THE MOLECULAR DEVELOPMENT OF MOTOR NEURONS. R.G. Kalb, S. Hockfield. *Dev. Biol.*, Vol. 162, pages 539-548; 1994.

SYNAPTIC ACTIVITY AND THE CONSTRUCTION OF CORTICAL CIRCUITS. L.C. Katz, C.J. Chatz. *Science*, Vol. 274, pages 1133–1138; 1996.

BIRDSONG AND HUMAN SPEECH: COMMON THEMES AND MECHANISMS. A.J. Doupe, P.K. Kuhl. *Ann. R. Neurosci.*, Vol. 22, pages 567–631; 1999.

EXPERIENCE-DEPENDENT DEVELOPMENT OF SPINAL MOTOR NEURONS. F.M. Inglis, K.E. Zuckerman, R.G. Kalb. *Neuron*, Vol. 26, pages 299–305; 2000.

SPACE FLIGHT AFFECTS MAGNOCELLULAR SUPRAOPTIC NEURONS OF YOUNG PREPUBERAL RATS: TRANSIENT AND PERMANENT EFFECTS. D. Garcia-Ovejero, J.L. Trejo, I. Ciriza, K.D. Walton, L. Garcia-Segura. *Dev. Brain Res.,* Vol. 130, pages 11–205; 2001.

SPACEFLIGHT INDUCES CHANGES IN THE SYNAPTIC CIRCUITRY OF POSTNATAL DEVELOPING NEOCORTEX. J. DeFelipe, J.I. Arellano, A. Merchan-Perez, M.C. Gonzákez-Albo, K. Walton, R. Llinás. *Cereb. Cortex,* Vol. 12, pages: 883–891; 2002.