Effects of prenatal spaceflight on vestibular responses in neonatal rats

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Ronca, April E., and Jeffrey R. Alberts. Effects of prenatal spaceflight on vestibular responses in neonatal rats. J Appl Physiol 89: 2318–2324, 2000.—Ten pregnant Norway rats (Rattus norvegicus) were flown for 11 days on board the NASA space shuttle from gestational day 9 (launch) until gestational day 20 (landing) of the rats’ 22-day pregnancy. After the birth of the pups, vestibular responses were analyzed from postnatal day (P) 0 until P5. In the first test, P0 neonates were supported on a platform in a side-lying position. Skyward head movements (i.e., movements performed against the gravity vector) were more frequent than head movements toward Earth in both flight and control neonates. In the second test, the contact-righting reflex, composed of stereotyped movements that rotate the body from supine to prone on a solid surface, was analyzed in P0 neonates. The frequency and latency of contact-righting responses did not differ in flight and control neonates. In the third test, vestibular head righting, with tactile and proprioceptive cues removed, was tested in neonates on P1, P3, and P5 by using a water-immersion test. Righting responses were observed less frequently in P1 and P3 flight neonates compared with controls. However, this deficit was transient, as evidenced by complete response recovery on P5. Collectively, these findings provide evidence for a selective disruption of vestibulomediated responses after prenatal exposure to spaceflight.

righting reflex; otolith; labyrinth; semicircular canals; development

PAST STUDIES OF VISUAL, auditory, olfactory, and tactile development (6, 10, 11, 18) have yielded extraordinarily important findings leading to the establishment of a fundamental tenant in developmental neuroscience: stimulation determines maturation. In the absence of modality-specific stimulation, immature sensory systems develop abnormally. Immature neuronal components, altered patterns of neural connectivity, and reduced sensitivity to stimulation within a given modality result from sensory deprivation (e.g., Ref. 10).

It is also true that the onset of function within a given sensory system precedes maturation (2, 9). There exists a sequentially fixed, highly conserved sequence of sensory ontogenesis in birds and mammals in which vestibular development begins before development of most, if not all, other modalities. The onset of vestibular function always occurs before birth or hatching (2), coincident with a period of rapid prenatal vestibular development (5, 7). In mouse (Mus) and rat (Rattus norvegicus), for example, neurovestibular development begins around gestational day (G) 9 with the formation of the otocyst and appearance of utriculi and sacculi on G14. Neurons within the vestibular nuclei differentiate between G11 and G15, and hair cell mitosis occurs from G14 to postnatal day (P) 2, peaking on G17. Afferent and efferent nerve endings first approach hair cells at G17 and G18, and morphological differentiation of the type I and type II hair cells begins around G19. Whereas vestibular hair cells do not attain mature appearances until the end of the first postnatal week and their connectivity continues to develop postnatally (4), responses to vestibular stimulation are first observed prenatally (14, 17). These considerations suggest that vestibular perturbations in utero, arising from gravity or produced by the pregnant mother's body movements, provide sensory input that is critical to the formation and establishment of mature, functioning gravistatic and acceleration systems (13, 16).

Of all the sensory modalities, the developing vestibular system has received the least attention. Unquestionably, this is due, in part, to the difficulty of depriving the vestibular system of the omnipresent stimulus of gravity. In the present study, we were able to use orbital spaceflight as the means of testing the hypothesis that the absence of the Earth-normal gravity stimulus during the prenatal period would “unload,” or reduce input to, the fetuses’ developing vestibular systems. Neural connectivity and onset of vestibular function were, therefore, challenged to form in the microgravity of space. We predicted changes in postnatal behavioral responses relying on prenatal vestibular input and function.

Ten pregnant rat dams spent 11 days in orbital spaceflight. The rats were launched on G9 and landed on G20, ~48 h before the pups' birth. The neonates' vestibular responses were studied during the first 5 days after birth (from P0 until P5). Using a battery of

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tests, we analyzed selected behavioral responses of neonates mediated predominantly by the otoliths and secondarily by the semicircular canals. The test battery was composed of 1) skyward head movements from the side-lying position, with and without head support, involving asymmetric stimulation of the labyrinths, 2) contact righting from supine to prone, involving symmetric stimulation of the labyrinths and tactile and proprioceptive stimulation, and 3) water-immersion righting, which consists of vestibular righting from supine to prone, involving symmetric stimulation of the labyrinths without tactile or proprioceptive input. None of the tests involves visual sensory input, as rat neonates’ eyes are sealed until at least P14. We selected tests previously used to reveal sequential ontogenetic changes in the sensory controls and motor output governing the emergence of head and body righting responses in the rat (12). In the present study, we utilized these tests to analyze the effects of prenatal spaceflight on postnatal vestibular responses during the early neonatal period.

METHODS

Subjects

Neonatal subjects were derived from 30 pregnant Sprague-Dawley rats (Taconic Farms, Germantown, NY) weighing between 165 and 205 g. Thirty-five foster dams were also used. Time-bred, nulliparous dams were shipped to Kennedy Space Center (KSC) on G2 (spermatozoa positive = G1). Animals were housed in a room with controlled lighting (6 AM to 6 PM) and temperature (~22°C). Pregnant rats on Earth were housed individually in standard vivarium cages (47 × 26 × 21 cm) with corn cob bedding material. Rat chow and water were available ad libitum. All animal procedures adhered to National Aeronautics and Space Administration (NASA) and National Institutes of Health Guide for the Care and Use of Laboratory Animals [DHEW publication no. (NIH) 85–23, revised 1985, Office of Science and Health Reports, DRINIH, Bethesda, MD 20205].

Treatment of Dams

Three groups of 10 pregnant rats and their offspring were used in the experiment. The experimental dams and offspring were treated identically throughout the study except for group differences, as described below. There were three relevant treatment groups.

Flight. Flight (Flt) dams (217 ± 9 g), housed in groups of five in standard animal enclosure modules (AEMs), the mid-deck-compatible habitats for rodents on the space shuttle, were exposed to launch, spaceflight, and landing.

Synchronous control. The synchronous control (Syn) dams (223 ± 18 g) were treated identically to Flt animals but were not exposed to launch, landing, or spaceflight. The Syn animals (n = 5) were housed in AEMs that were, in turn, contained within the orbital environment simulator chamber at KSC. Animals in the Syn group were 24-h delayed relative to the Flt group. This allowed time for shuttle environmental conditions (i.e., temperature, humidity, and lighting) to be downlinked and mimicked in the Syn group.

Vivarium control. The vivarium control (Viv) dams (223 ± 3 g) were housed in standard vivarium cages and maintained in a colony room at KSC throughout the experiment to provide normative, baseline data.

Maternal Surgeries

The dams in all three experimental conditions sustained two surgical procedures. A report describing the background, rationale, and efficacy of the procedure can be found elsewhere (3). On G7, 2 days before flight, each dam sustained a surgical laparotomy to confirm pregnancy and establish the number of implantation sites. Dams were selected for inclusion in the study only if a minimum of five embryos populated each of their paired uterine horns. On G20, immediately after recovery from the space shuttle, dams sustained a unilateral hysterectomy, which provided fetal tissue to other investigators selected by NASA for studies concerned primarily with prenatal anatomic development (e.g., Ref. 8). This experimental protocol yielded fetuses from each of the 30 dams comprising the Flt, Syn, and Viv groups. After the unilateral hysterectomy, dams were permitted to recover and deliver vaginally, thereby providing neonates for postnatal behavioral analyses.

Surgical Laparotomy of Pregnant Dams

A surgical laparotomy was performed on each dam under aseptic conditions on G7, the earliest day on which implantation sites (decidual swellings) can be reliably visualized. Briefly, each dam was anesthetized with isoflurane (IsoFlo, Abbott Labs, North Chicago, IL) vapor with the use of a non-rebreathing rodent anesthesia unit (Viking Products, Medford Lakes, NJ). The fur overlying the abdomen of the anesthetized rat was shaved, the skin was cleansed with an antiseptic and alcohol, a veterinary opthalmic ointment was applied, and an antibiotic and analgesic mixture (10,000 IU, Microcillin-AG, Anthony Products, Arcadia, CA; and 10 mg/kg, butorphanol tartrate, Fort Dodge Labs, Fort Dodge, IA) was given by subcutaneous injection. An incision was made, beginning ~2 cm cranial to the pubis and extending cranially 2–3 cm. Each uterine horn was gently grasped between the decidual swellings and externalized for close visual inspection, and counts were made of implantation sites, which were recorded. The uteruses were carefully reinserted into the abdominal cavity, after which interrupted sutures were used to close the peritoneum and muscle layer. The overlying skin was then closed with 9-mm wound clips. The entire procedure lasted 10 min.

On G8, the Flt and Syn dams were housed in groups of five within an AEM, which is NASA’s flight cage for group-housed adult rodents. The animal chamber within the AEM is a stainless steel mesh cage, ~23.5 × 35.6 × 21.6 cm (in Earth-gravity orientation). Food was available in the form of food bars, each ~2.5 × 2.5 × 20 cm, attached to the walls of the AEM. These food bars are fabricated from a commercial diet (Teklad Diets, Madison, WI) and are nutritionally complete and resistant to spoilage. Water was available from any four lixit valves that protruded from a stainless steel box (21 × 11.1 × 15.2 cm) within the AEM. With the water system and food bars in place, the AEM is a compact volume for five pregnant rats. Airflow through the AEM is controlled by external fans that create a near-laminar flow moving from ceiling to floor (in Earth-normal orientation); the airstream moves through the waste tray, which contains activated charcoal and absorbent filter. On G9, space shuttle Atlantis launched, carrying the 10 pregnant Flt dams into low-Earth orbit.

Unilateral Hysterectomy of Pregnant Dams

The purpose of the unilateral hysterectomy was to provide a sampling of fetuses soon after return from spaceflight. The
dams in all three experimental conditions underwent unilateral hysterectomy. Within 2 h after recovery from spaceflight, the first Flt dam was anesthetized. Anesthesia and surgical preparations were identical to those described for the laparotomy procedure on G7. The uterine horns were exteriorized by extending the midventral abdominal incision. The uterine horn removed was alternated across rats in each treatment group. The horn was ligated cranially and caudally with braided silk (20 Ethicon, Somerville, NJ) and then excised. The incision was closed by employing procedures used for the laparotomy. The unilateral hysterectomized dams were single housed and placed in a colony room. Daily records of food bar consumption, water intake, and body weight were maintained for the remainder of the study. Pregnancies were permitted to go to term.

**Treatment of Neonates**

After parturition, neonates in each condition were weighed, sexed, and individually identified by using a nontoxic felt-tip marker. (The markings were refreshed every day or two.) Neonates in each condition were then given to foster dams that had delivered their own litter within 12 h of the experimental dams. This procedure was implemented to avoid secondary effects transmitted to postnatal offspring via postnatal maternal behavior and physiology. Foster litters were adjusted to 10 neonates each by retaining some of the foster dams’ original neonates. The newly constructed litters were maintained in the colony for the remainder of the study.

**Testing Procedure and Methods of Behavioral Recording**

Neonates were given a different test on each day from P0 until P5: skyward head lifts and contact righting on P0, and water-immersion righting on P1, P3, and P5. During testing, neonates were removed from the nest as a litter and placed in a weigh boat in a heated incubator (33°C). Individual body weights were measured, and identification markings were refreshed before the neonates were returned to the dam. All of the behavioral tests were videotaped on high speed (1/1,000 s) with an 8-mm camcorder (Nikon) under bright lighting. This system provided high-resolution, blur-free frames for analysis of movements during slow-motion tape review.

**Skyward Head Movements from the Lateral Position**

Each P0 neonate was tested by using a small plastic trough (8 cm long × 3 cm in diameter) lined with a thin layer of felt. On P0, each neonate was positioned on its right side and tethered with felt strips within the body of the trough. The portion of the trough supporting the head (3 cm) was discontinuous with the main body of the apparatus and held in place with a spring assembly such that the two segments of the trough were closely aligned and the discontinuity between segments was imperceptible. Each neonate was videotaped for 30 s, which, after removal of the head support, continued for an additional 30 s to determine the head response direction (e.g., lifts vs. lowers). The procedure was immediately repeated, and the neonate then returned to its litter.

**Contact Righting from Supine to Prone**

On P0, neonates were placed in the supine position on a foam pad (30 × 23 cm) with a mirror angled at the rear of the foam to capture an on-camera view of the neonate from each side. Each neonate was held gently to the pad with the experimenter’s fingers around the pelvic girdle and head and then released. The following rating scale was used to quantify righting success. A score of 0 was assigned if the animal made no attempt to right itself; a score of 1 if a “corkscrew” pattern was observed but righting was not achieved; and a score of 2 for complete righting. Righting was considered to be successful if the neonate achieved the prone position with both forelimbs in contact with the foam surface (i.e., achieved a score of 2). Latency to achieve this prone position on release by the experimenter was also measured from the videotapes. The test was discontinued on successful righting (assignment of a score of 2) or after a 2-min period.

**Water-Immersion Righting from Supine to Prone**

On P1, P3, and P5, each neonate was positioned in the supine position in a clear container (1-gallon aquarium) filled with heated (38°C) water, 2–3 cm beneath the surface of the water, and then released. The coding system used for contact righting was adapted for use in this test. Successful vestibular righting was scored if the neonate turned fully from supine to prone (both forelimbs facing the base of the tank) before reaching the bottom of the tank (~10 cm). Response latency was measured from the time of release to the achievement of righting or reaching the tank base, whichever occurred first. This “water-immersion righting” response is typically absent at birth (P0) but matures over the period from P1 to P5.

**Data Analysis**

Videographic data were coded by trained scorers during slow-motion playback of the videotapes. Because multiple offspring do not represent multiple, independent observations for statistical purposes (1), individual data were expressed as litter means and analyzed by using ANOVA. If significance was found, group differences were identified by using the Newman-Keuls test. Nominal data were analyzed by using a χ² test.

**RESULTS**

Parturition occurred at the expected time (on G22 and G23) in the majority of dams. Two dams in the Flt group and one in the Syn group had not delivered by 1500 on G23. Neonates from these dams were delivered by cesarean section under brief isoflurane anesthesia. Of the 30 litters that were born, one or two neonates from each of 24 litters were available for our analyses (the remaining litters and neonates were assigned to another NASA-funded investigator). The data presented are composed of a total of 34 neonates from 24 dams (Flt, n = 8; Syn, n = 7; Viv, n = 9).

**Neonatal Body Weights**

Body weights of the Flt and Syn neonates were not statistically different on any day between P0 and P5, and body weights of Syn and Viv neonates were not statistically different on any day between P0 and P5 (Table 1). However, Flt neonates weighed significantly less than the Viv neonates on each day except P2 [condition F(2,21) = 3.69; P < 0.04; condition × day F(10,105) = 2.77; P > 0.05; Newman-Keuls, P > 0.05].

**Skyward Head Responses from the Lateral Position**

During the initial 30-s period with the head support in place, strong lateral lifts were observed, approach-
ing 90° from lateral. Active responses were made in the up, but not down, direction for the Flt, Syn, and Viv conditions. With head support in place, the frequency of head lifts observed in Flt neonates was comparable to the frequency of lifts observed in Syn and Viv controls [response frequency: trial 1, Flt, 9.3 ± 4.5 (SD); Syn, 10.2 ± 4.5; Viv, 12.6 ± 7.7; F(2,21) = 0.69, not significant (NS); trial 2, Flt, 3.8 ± 4.2; Syn, 8.7 ± 9.0; Viv, 4.3 ± 1.6; F(2,21) = 2.1; NS]. When the head support was removed (Fig. 1), Flt neonates continued to lift their heads at the same frequency as before, whereas neonates in both control groups inhibited responding [change from baseline: trial 1, Flt, 2.7 ± 6.3 (SD); Syn, −6.6 ± 9.1; Viv, −2.7 ± 4.8; F(2,21) = 3.6, P < 0.05, Newman-Keuls P < 0.05; trial 2, Flt, 6.9 ± 8.2; Syn, −4.0 ± 7.0; Viv, −2.72 ± 1.6; F(2,21) = 5.8, P < 0.01, Newman-Keuls P < 0.05].

Contact Righting from Supine to Prone

The characteristic response of the newborn rat when placed on its back on a solid surface is to assume a U posture followed by rotation of the head, neck, and shoulders with forepaw support and then righting of the body (Fig. 2, top). All of the neonates attempted to right themselves (i.e., none received a score of 0). P0 Flt neonates successfully righted themselves (i.e., achieved a score of 2) as often as did Syn and Viv neonates [Fig. 2, bottom: Flt, Syn, χ² (1) = 3.4, NS; Flt, Viv, χ² (1) = 0.0, NS; Syn, Viv, χ² (1) = 3.4, NS]. Table 2 shows the percentage of neonates assigned each score during contact righting. Similar proportions of animals in the three groups received scores of either 1 (incomplete righting) or 2 (successful righting).

Response latencies, as measured by the amount of time required to reach the prone posture (i.e., achieve a score of 2), were also similar in Flt, Syn, and Viv animals [Flt, 76.0 ± 57.7 (SD) s; Syn, 76.2 ± 45.3 s; Viv, 65.2 ± 38.9 s; F(2,21) < 1, NS].

Vestibular Righting from Supine to Prone in the Absence of Tactile and Proprioceptive Cues

When immersed in a heated water bath and released, P3 neonatal rats rotate their bodies from supine to prone before reaching the bottom of the tank (Fig. 3, left), achieving a score of 2. In contrast, Fig. 3, right, shows sample video image of a nonresponder, a neonate that made no righting attempts before reaching the bottom of the water bath. This pattern was observed more often in Flt neonates compared with

Table 1. Postnatal body weights from P0 until P5 of offspring derived from flight, synchronous, and vivarium dams

<table>
<thead>
<tr>
<th>Condition</th>
<th>P0</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flight</td>
<td>6.1 ± 0.1a</td>
<td>7.3 ± 0.2a</td>
<td>8.1 ± 0.2a</td>
<td>9.0 ± 0.3f</td>
<td>10.7 ± 0.4h</td>
<td>12.2 ± 0.5f</td>
</tr>
<tr>
<td>Synchronous</td>
<td>6.3 ± 0.2b,c</td>
<td>7.1 ± 0.3c</td>
<td>8.5 ± 0.3d</td>
<td>9.7 ± 0.4e</td>
<td>11.6 ± 0.5h,i</td>
<td>12.9 ± 0.6k</td>
</tr>
<tr>
<td>Vivarium</td>
<td>6.7 ± 0.2bc</td>
<td>7.8 ± 0.3b</td>
<td>9.3 ± 0.3c</td>
<td>10.5 ± 0.03e</td>
<td>12.3 ± 0.4j</td>
<td>14.0 ± 0.6k</td>
</tr>
</tbody>
</table>

Values are means ± SD in grams, expressed as litter averages; n = 34 neonates from 24 dams (8 flight, 7 synchronous, 9 vivarium). P0–P5, postnatal days 0–5. Means with different superscripts are different (P < 0.05).
Syn neonates. Figure 4 shows the percentage of neonates in each group that successfully righted themselves (i.e., achieved a score of 2) on P1, P3, and P5. On P1, a fewer proportion of Flt neonates successfully righted themselves compared with Syn or Viv neonates, as measured by the assignment of a score of 2 \[\text{Flt, Syn, } \chi^2 (1) = 6.1, P < 0.01; \text{Flt, Viv, } \chi^2 (1) = 12.5, P < 0.001\]. Similar proportions of Syn and Viv neonates showed complete righting responses \[\text{Fig. 3; Syn, Viv, } \chi^2 (1) = 1.3; \text{NS}\]. On P3, fewer Flt neonates responded compared with Syn and Viv animals \[\text{Flt, Syn, } \chi^2 (1) = 27.7, P < 0.0001; \text{Flt, Viv, } \chi^2 (1) = 50.0; P < 0.0001; P < 0.001\], whereas the two control conditions did not differ from one another \[\text{Syn, Viv, } \chi^2 (1) = 1.1; \text{NS}\].

Table 2 shows the percentage of neonates assigned a score of 0, 1, or 2 during analysis of the video images taken during water righting. On P1, twice as many Flt neonates compared with Syn neonates made no attempt to right themselves in the bath. Analyses of response latencies on P3 indicated that Flt neonates took significantly longer to achieve the prone position relative to Syn and Viv neonates \[\text{Flt, } 477 \pm 151 \text{ ms; Syn, } 267 \pm 99 \text{ ms; Viv, } 343 \pm 124 \text{ ms; } F(2,21) = 5.7; P < 0.01; \text{Newman-Keuls test, } P < 0.05\].

DISCUSSION
Prenatal exposure to spaceflight, coincident with the period during which the neurovestibular apparatus first takes form and begins to function, altered postnatal vestibular responses of microgravity-exposed fetuses. The specific tests we employed were based on a previous analysis of sequential changes in sensory controls and motor output in neonatal rats during ontogeny of the righting response (12). Taken together, the findings of this study suggest that exposure to spaceflight during the latter half of gestation is associated with modality-specific effects on vestibular function. We review, in detail, the results of each test alone, as well as their integrative value.
Skyward Head Responses from the Lateral Position

The head-lift responses observed on P0 were clearly movements against the gravity vector, indicating that the P0 Flt neonates’ vestibular systems were functioning. The skyward response is present from about P0 to P5 in the rat, antedating characteristic head rotations to the upright position (12). It has been noted, however, that the skyward response is potentiated by asymmetric tactile stimulation of the shoulders. The lack of response inhibition by Flt neonates when the surface is removed may indicate augmented sensitivity to vestibular cues in the neonates that underwent a substantial proportion of the gestation during spaceflight.

Contact Righting from Supine to Prone

Righting on a surface is a predominant response in the behavioral repertoire of the neonatal rat. After prenatal spaceflight, neonates performed well in the contact-righting test, achieving identical probabilities of success and similar latencies to achieve the prone position compared with ground controls. There were no differences across groups in the manner in which neonates in the different conditions righted themselves. The typical sequence of movements shown during righting by neonates was observed (Fig. 2, top). Together, these findings suggest that spaceflight and exposure to microgravity during gestation had no effect on this vestibular-mediated response. However, added cues associated with the solid surface (tactile and proprioceptive) may have contributed to the execution of the response. We reasoned that postflight changes in vestibular function might go undetected in analyses of contact righting alone because tactile and proprioceptive input could help the neonates compensate for reduced vestibular sensitivity. Therefore, righting within a water bath, which relies solely on vestibular input, was also studied.

Vestibular Righting from Supine to Prone in the Absence of Tactile and Proprioceptive Cues

The water-immersion test revealed clear response deficits among a substantial proportion of the neonates that underwent prenatal development in space. On P1, the day on which the response is generally incomplete in the neonatal rat, twice as many Flt neonates made no attempt to right themselves compared with controls. Similar proportions of animals made righting attempts, but fewer Flt neonates succeeded in righting before reaching the bottom of the tank.

In general, the Flt animals did not perform the response well on P3. Whereas successful righting was observed in >90% of the control animals, only 60% of the Flt animals were observed to right themselves completely. In addition, 10% of the Flt neonates (as compared with 0% of the controls) made no righting attempts. The pattern of response latencies, significantly longer in the Flt condition, supports the results of the qualitative analysis. These findings suggest that a proportion of the Flt animals was insensitive to the vestibular cues. However, this deficit was transient, as evidenced by the complete success with which Flt neonates responded on P5, matching the performance of the control subjects.

The nature of the response deficit cannot be ascertained with certainty based on the results of this study alone. As evidenced by the neonates’ performance during the contact-righting test, Flt pups were capable of the motoric sequence of righting themselves, thus the deficiency was not movement based. In addition, 60% of the Flt neonates did show the complete water-righting response. The observation that the water-righting responses of the P5 Flt animals were indistinguishable from those of controls suggests developmental recovery or readaptation in the presence of Earth-normal gravitational cues. The pups’ postflight changes reflect a shift from in-flight profiles of stimulation to those imposed by a 1-G Earth-normal environment.

Microgravity exposure during gestation appears to have selectively diminished vestibular responsivity around the time of developmental emergence of this sensory modality. This view does not exclude the possibility of a “critical period” in vestibular system development; however, it is important to recognize that our interpretation is presently limited by the window provided by the spaceflight mission and restrictions on the number and kinds of controls that can be incorporated in this work. Although the results are suggestive of developmental change, at this time, we cannot claim with certainty that these are developmental effects. The Flt neonates’ pattern of developmental delay could be due to stress effects associated with launch, spaceflight, and landing. If different ages and/or different durations are studied in future experiments and more extensive controls are utilized, we will be in a far better position to speak on the mechanisms operating in these findings of apparent developmental delay.

Considered in concert with findings from a different spaceflight mission, we can speculate that the present results do suggest a developmental effect. We previously postulated (15) that prenatal exposure to microgravity unloads the fetuses’ otoliths and hyperstimulates the semicircular canals. We tested the hypothesis that the fetuses’ responses to angular stimuli would be greater after spaceflight and would be associated with movements of the pregnant mother’s body in the weightless space environment. We found evidence for this supposition. Prenatal (G20) rat fetuses exposed to spaceflight during gestation showed precocial responses to tilt stimulation that were significantly different from those of Syn animals. We conducted a kinematic analysis of the dams’ in-flight behavior using daily video recordings of the dams on the shuttle. The most dramatic difference found in the behavior of Flt dams relative to controls was the number of times the dams moved by rolling. Flt dams displayed about seven times more rolling movements than did controls. Accelerations of pitch and yaw were equivalent across groups. Altered accelerations (a selective increase in rolling movements) delivered to the fetuses by the
weightless dams’ behavior could contribute to the perinates’ precocial responses to tilt and rotation. The findings of the present experiment fit well with our hypotheses of prenatal vestibular development in microgravity. The observation of augmented skyward-lifting responses in F1t neonates after removal of tactile inputs may be due to increased sensitivity of gravitational cues in these subjects, mediated by changes in the development of the semicircular canals and/or their projections. Diminished righting of F1t neonates in the water-immersion test is consistent with developmental changes that would be expected after unloading of the prenatal otoliths during formative periods of vestibular system development. This interpretation of our functional results receives support from anatomic studies. Fritzsch and Bruce (8) reported that utricular and saccular axons of microgravity-exposed fetuses were largely unbranched, generally ending in growth cones, whereas corresponding axons in controls showed elaborate branching. In contrast, facial sensory neurons of microgravity-exposed fetuses had exuberant branches to the utricle that were virtually absent in controls. Taken together with the results of the present study, these findings suggest that gravitational input present during early development may shape the neural architecture and function of the vestibular system.

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