Effects of Hypergravity Exposure on the Developing Central Nervous System: Possible Involvement of Thyroid Hormone

ELIZABETH M. SAJDEL-SULKOWSKA,*,†,[1] GUI-HUA LI,† APRIL E. RONCA,‡ LISA A. BAER,§
GREGORY M. SULKOWSKI,|| NORIYUKI KOIBUCHI,¶ AND CHARLES E. WADE‡

*Department of Psychiatry, Harvard Medical School and †Brigham and Women's Hospital, Boston, Massachusetts 02115; ‡Life Sciences Division, NASA/Ames Research Center, Moffett Field, California 94035; §Lockheed Martin Engineering and Sciences, Moffett Field, California 94035; ||Program in Cognitive Neuroscience, Harvard University, Cambridge, Massachusetts 02138; ¶Department of Physiology and CREST, Gunma University School of Medicine, Maebashi, Gunma 371–8511, Japan

The present study examined the effects of hypergravity exposure on the developing brain and specifically explored the possibility that these effects are mediated by altered thyroid status. Thirty-four timed-pregnant Sprague-Dawley rats were exposed to continuous centrifugation at 1.5 G (HG) from gestational Day 11 until one of three key developmental points: postnatal Day (P) 6, P15, or P21 (10 pups/dam: 5 males/5 females). During the 32-day centrifugation, stationary controls (SC, n = 25 dams) were housed in the same room as HG animals. Neonatal body, forebrain, and cerebellum mass and neonatal and maternal thyroid status were assessed at each time point. The body mass of centrifuged neonates was comparatively lower at each time point. The mass of the forebrain and the mass of the cerebellum were maximally reduced in hypergravity-exposed neonates at P6 by 15.9% and 25.6%, respectively. Analysis of neonatal plasma suggested a transient hypothyroid status, as indicated by increased thyroid stimulating hormone (TSH) level (38.6%) at P6, while maternal plasma TSH levels were maximally elevated at P15 (38.9%). Neither neonatal nor maternal plasma TH levels were altered, suggesting a moderate hypothyroid condition. Thus, continuous exposure of the developing rats to hypergravity during the embryonic and neonatal periods has a highly significant effect on the developing forebrain and cerebellum and neonatal thyroid status ($P < 0.05$, Bonferroni corrected). These data are consistent with the hypothesized role of the thyroid hormone in mediating the effect of hypergravity in the developing central nervous system and begin to define the role of TH in the overall response of the developing organism to altered gravity.

Key words: hypergravity; rat; cerebellum; development; thyroid hormone [Exp Biol Med Vol. 226(8):790–798, 2001]

Although the effects of hypergravity on the central nervous system (CNS) are well established, the mechanisms involved in these changes are not completely understood. In the present study we explored the possibility that the status of the thyroid hormone (TH) is compromised by altered gravity and in turn affects CNS development. Such a possibility of the involvement of the TH in the physiological response of humans and animals to altered gravity has been suggested by the results of both hypo- and hypergravity experiments. Hypothyroidism has been observed in astronauts (1) and rats exposed to microgravity (2–4). A transient change in TH levels was observed in rat dams exposed to hypergravity during the 48 hr of peripartum (5). It is likely that changes in plasma TH levels in response to altered gravity affect the function of a number of systems, including the CNS.

Because up to 70% of astronauts experience some form of motion sickness and disturbances in motor coordination and movement (6), most of the animal studies to date have targeted the response of the peripheral vestibular system to altered gravity (7). A number of ground-based experiments employing hypergravity generated in a large-radius horizontal centrifuge (8) have suggested that hypergravity is a good model to evaluate potential effects of reduced gravity during space flights. Altered function of the vestibular system has been observed in adult rats (9) and hamsters raised under hypergravity (10). Since even a short exposure to altered gravity encountered by astronauts (6) or adult animals (7, 8) affects CNS function, prolonged exposure to altered gravity during embryonic and early neonatal development is ex-

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This work was supported primarily by NASA (grant no. NCC2-1042 to E.M.S.S.). Additional support was provided by NASA (grant nos. 121-10-30, 121-10-40, 121-10-50, and 121-40-10).

[1] To whom requests for reprints should be addressed at Department of Psychiatry, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115. E-mail: Esulkowska@rics.bwh.harvard.edu

Received September 1, 2000.
Accepted April 17, 2001.

0037-9727/01/2268-0790$15.00

pected to have much more dramatic and long-lasting consequences. As the possibility of long-term space travel and habitation is becoming a reality, understanding the development of the CNS under microgravity is increasingly important.

In the present study we explored the possibility of utilizing the developing rat cerebellum in studying global changes in the CNS developing under altered gravity. A number of factors make the cerebellum a particularly attractive system in which to study the mechanism of hypergravity-induced changes in the CNS. Altered gravity compromises the functions of the cerebellum, which, as part of the vestibular system, receives input relevant to gravitational level and body position. In the rat, the cerebellum develops postnatally between birth and weaning, and during that period, all the developmental processes of cell proliferation, migration, and differentiation can be addressed. Furthermore, regulation of cerebellar development in the rat has been extensively studied, and the critical role of the TH is well recognized. However, despite the fact that the relationship between thyroid status and CNS development has been well established, neither cerebellar development, thyroid status, nor the relationship between the two under altered gravitational conditions has previously been addressed.

Cerebellar development is regulated by thyroid hormones (3,5,3′-L-triodothyronine, T3; 3,5,3,5′-L-tetraiodothyronine, T4; TH; [11, 12]). The active form, T3, depends to a great extent on intracellular generation from T4 (13). Deficiencies in plasma TH levels result in decreased TH in the neonatal brain (14) and lead to cytoarchitectural abnormalities and the mental retardation known as cretinism in humans (15, 16). The results of several studies involving rats (17–20) pointed out that during gestation through postnatal Day 15 (P15), brain development is particularly vulnerable to TH deficiency. Propylthiouracil-(PTU) induced hypothyroidism following exposure before P15 has a dramatic effect on the animal's growth and cerebellar development, manifested in morphological abnormalities and by a reduction in size (17). PTU administration following P15 does not affect cerebellar development. Furthermore, even moderate hypothyroidism during gestation, characterized by increased plasma TSH but normal T4, has a negative effect on the availability of T4 to fetuses (21) and is likely to affect brain development. The hypothyroid rat model has been used in our studies to provide us with important reference points while we explore the potential involvement of TH in the hypergravity response (22).

On the basis of independent observations of changes in both the CNS and TH in response to altered gravity and of the well-recognized role of TH in the regulation of cerebellar development, we formulated the hypothesis that the effect of hypergravity on cerebellar development is mediated by altered thyroid status. As a first step in testing this hypothesis, we examined the effect of perinatal hypergravity (1.5 G [HG]) exposure, on the developing CNS and on the thyroid status of rat neonates and dams. Specifically, forebrain and cerebellar size and plasma TSH, T3, and T4 were compared in rats exposed to HG and stationary controls (SC, 1.0 G) at critical neonatal ages: P6 (neonatal/peak of cell proliferation), P15 (preweaning/time of cell migration), and P21 (weaning/time of neuronal differentiation). The results, presented here, suggest that hypergravity-induced changes in the developing cerebellum are associated with altered thyroid status.

Materials and Methods

Subjects. All procedures were reviewed and approved by Institutional Animal Care and Use Committees at Harvard Medical School and at NASA Ames Research Center. Eighty-three primiparous timed-pregnant Sprague-Dawley dams were shipped from Taconic Farms (Germantown, NY) to NASA Ames Research Center (ARC) at gestational Day (G) 2 (spermatozoa observed in a vaginal lavage at G1). Dams were individually housed under standard vivarium conditions (12:12-hr light:dark cycle, with lights on at 0600 hr and off at 1800 hr, at 21° to 24°C). Standard laboratory rat chow and water were available *ad libitum*. Chow was placed in both the food rack and the cage bottoms from G8 throughout the remainder of the experiment to facilitate access to food for parturient dams and preweanling/weanling neonates.

Procedures. Before centrifugation, animals were handled and weighed daily, thereby acclimatizing them to the handling procedures during the experiment. Three days before centrifugation, at G8, dams were weight-matched and assigned to either HG ($n = 32$ litters) or SC ($n = 25$ litters) conditions and were adapted to the NASA ARC 24-ft centrifuge facility by being placed in the centrifuge rotunda. Both HG and SC dams were placed 1 per cage in regular shoebox-type maternity cages (119 × 66 × 21 cm) and loaded were 2 or 4 cages per cab; all animals were exposed to the same environment. At P1, all neonates were pooled and randomly assigned to dams within each experimental group (10 neonates/dam; 5 males and 5 females). During the subsequent days, the number of SC neonates was adjusted to match the HG group by removal of pups to compensate for a possible effect of litter size. Dams' body weights were measured daily from G2 until P21, and litter weights were measured daily after birth.

Centrifugation. At G11, continuous centrifugation (15.94 RPM, 7.3-m diameter, resultant 1.5 G) was initiated with brief (<1 hr) daily stops for animal health checks and data collection. This device and associated procedures have been used previously in numerous experiments investigating physiological and behavioral responses of rats to centrifugation (5, 8, 23). The cabs housing rats were suspended from the radial arms of the centrifuge by a yoke, allowing them to swing out during rotation. The HG animals were thus subjected to the resultant gravitational and centrifugal force of 1.5 G in the normal direction (i.e., perpendicular to the cage floor).

Tissue Collection. At P6 (HG, $n = 16$, SC, $n = 12$), P15 (HG, $n = 7$; SC, $n = 6$), and P21 (HG, $n = 8$; SC, $n = 7$), dams and offspring from each group were removed from their cabs and weighed individually. Dams and neonates were euthanized by live decapitation without anesthesia within 3 hr of stopping of the centrifuge. Trunk blood was collected from both dams and neonates for TH and TSH analysis. The samples were centrifuged for 10 min at 4°C. The resulting plasma was stored at −80°C until analyzed for T3, T4, and TSH. The neonatal brains were then rapidly removed, cerebella were dissected out, and both forebrain (brain-cerebellum) and cerebellar tissue were weighed.

Plasma T3, T4, and TSH Content. Plasma TSH, total T3, and total T4 were measured by commercial assays according to the manufacturer's instructions. Coefficients of variation for the TSH assays (Amersham Pharmacia Biotech, Piscataway, NJ) were 9.6% within assay and 4.9% between assays. For total T3 and total T4 (Diagnostic Products Corporation, Los Angeles, CA), the within-assay variability was 5.9% and 8.1%, and the between-assay variability was 6.6% and 7.7%, respectively. For the P15 and P21 neonates, equal volumes of plasma were used from littermates to produce pooled representative samples. For the P6, neonates equal volumes of plasma were pooled from two litters.

Statistical Analysis. An initial three-way ANOVA was performed with gravitational condition, age, and either body, forebrain, or cerebellum mass as a factor to determine whether the effect of hypergravity on the CNS is independent of general effect on body mass. The results of this analysis indicated a different effect of gravity on each of these parameters. Consequently, for separate analysis of body, forebrain, and cerebellar mass, a two-way ANOVA was run on litter averages, following log transformation of data to normalize the distribution, to determine the relationship between gravitational condition and age. If a statistically significant interaction was found between age and gravitational condition, then two-sample t tests were carried out at each age and the values were adjusted for multiple comparison by the Bonferroni correction. All values are reported as means ± SEM. Simple regression analysis of relationships also was performed with the same software. For all statistical tests, the 0.05 level of confidence Bonferroni corrected was accepted for statistical significance.

Results

Effect of Hypergravity on Pregnant Dams and Pregnancy Outcome. During pregnancy (G11 to G21), HG dams consumed less food (208.6 ± 3.06 g) than did SC dams (260.7 ± 3.85 g, DF = 57, $t = -10.734$, $P = 0.0004$, Bonferroni corrected) and gained less weight (87.8 ± 1.99 g) than did SC dams (108.35 ± 2.89g, DF = 57, $t = -6.031$, $P < 0.0004$, Bonferroni corrected). It is possible that this difference reflects the decrease in food intake observed in HG dams during the first 3 days of centrifugation. The relative food consumption (adjusted to body mass) was decreased from 0.97 ± 0.016 g/g body wt in SC dams to 0.87 ± 0.018 g/g body wt in HG dams (DF = 57, $t = -3.766$, $P = 0.0016$, and the relative gain in body mass was also decreased from 0.402 ± 0.009 g/g body wt in SC dams to 0.365 ± 0.009 g/g body wt in HG dams (DF = 57, $t = -2.89$, $P = 0.022$).

A greater proportion of HG dams (61.8%) than SC dams (20%) gave birth at G23, but the average number of neonates per litter was not affected (HG, 11.29 ± 0.43; SC, 11.88 ± 0.59; DF = 57, $t = -0.87$, $P = 0.42$).

Effect of Hypergravity on Neonates. Neonates born to HG mothers were smaller at birth at 6.28 ± 0.12 g/neonate than those born to SC mothers at 7.02 ± 0.14 g/neonate (DF = 57, $t = -4.094$, $P < 0.0001$). At P1, to minimize the litter variability, all neonates within the group were pooled and randomly distributed to dams within the group, with an equal female-to-male ratio. Following cross-fostering, the average litter body mass at P1 was 6.57 ± 0.046 g/neonate in the HG group compared with 7.67 ± 0.052 g/neonate in the SC group (DF = 56, $t = -15.862$, $P < 0.0001$). The overall attrition between P1 and P5 was 0.4% ± 0.4% per litter and 10.91% ± 2.06% per litter in SC and HG neonates, respectively (DF = 56, $t = 4.388$, $P < 0.0001$). No further losses beyond that point were observed.

Effect on Body, Forebrain, and Cerebellar Mass Are Independent of Each Other. The results of initial three-way ANOVA, with gravitational condition, age, and either body, forebrain or cerebellum mass as a factor suggested that the effect of hypergravity on neonatal CNS is independent of the general decrease in body size of HG neonates and that changes in the two components of the CNS are also independent of each other. Consequently, the effects of hypergravity on neonatal body mass, forebrain, and cerebellum weight are presented separately.

Body Size of Hypergravity-Exposed Neonates. Although all animals gained weight, at P6 the body mass of the HG neonates (Fig. 1) was decreased by 24.4% from 16.64 ± 0.238 g/neonate in the SC group to 12.56 ± 0.245 g/neonate in the HG group (DF = 26, $t = -11.635$, $P < 0.0003$). The difference in the neonatal body mass (16.8% reduction) was also significant at P21; the average neonatal mass was 58.96 ± 1.712 g/neonate in SC group as compared with 49.42 ± 1.322 g/neonate in HG group (DF = 13, $t = -4.458$, $P = 0.0018$). At P15 (11.95% reduction) the difference approached significance (HG = 32.73 ± 1.145 g/neonate, SC = 37.17 ± 1.215 g/neonate; DF = 12, $t = -2.626$, $P = 0.066$). The relative neonatal body mass (adjusted to forebrain mass) was decreased at P6 by 10.2% (HG = 24.26 ± 0.282, SC = 27.02 ± 0.310; DF = 26, $t = -6.535$, $P < 0.0003$) but was not affected at P15 or P21.

Brain Mass of Hypergravity-Exposed Rat Neonates. The neonatal forebrain mass (Fig. 2A) was reduced at P6 from 0.616 ± 0.005 g/neonate in the SC group to 0.518 ± 0.008 g/neonate in the HG group (DF = 26, $t = -9.182$, $P < 0.0003$), with a smaller reduction from 1.166 ± 0.012 g/neonate in SC group to 1.086 ± 0.013 g/neonate in the HG group at P15 (DF = 12, $t = -4.604$ $P = 0.0018$), and from

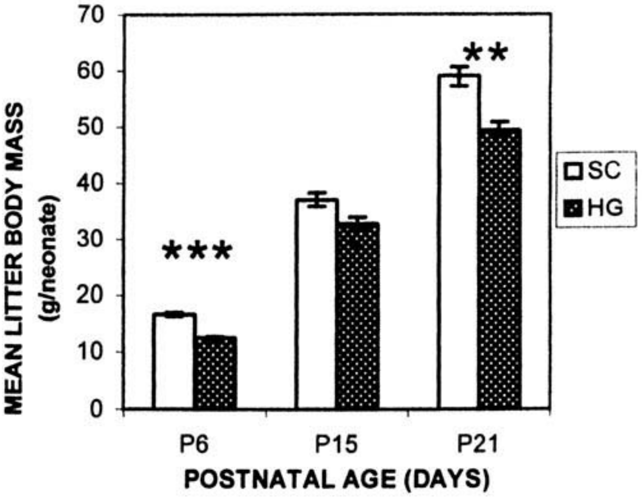

Figure 1. Effect of hypergravity on neonatal body mass. Data represent mean and SEM of litter body mass (grams per neonate). Each group contained 6 to 8 litters with 6 to 10 neonates per litter. The difference between HG and SC is indicated when significant (**$P < 0.005$, ***$P < 0.0005$).

1.326 ± 0.015 g/neonate in SC group to 1.212 ± 0.008 g/neonate in the HG neonate at P21 (DF = 13, $t = -6.917$, $P < 0.0003$). When the forebrain size was adjusted to body size (grams of tissue per gram of body weight neonate), there was a relative 10.8% increase in forebrain mass at P6 (HG = 0.041 ± 0.0005, SC = 0.037 ± 0.0004; DF = 26, $t = -6.195$, $P < 0.0003$) and no significant difference at P15 (HG = 0.033 ± 0.001, SC = 0.031 ± 0.001; DF = 12, $t = 1.388$, $P = 0.5712$) or at P21 (HG = 0.025 ± 0.001, SC = 0.023 ± 0.001; DF = 13, $t = 2.184$, $P = 0.1437$).

The cerebellar size (Fig. 2B) was reduced at P6 from 0.043 ± 0.001 g/neonate in the SC group to 0.032 ± 0.0005 g/neonate in the HG group (DF = 26, $t = -10.60$, $P < 0.0003$) with a nonsignificant reduction at P15 from 0.157 ± 0.002 g/neonate in the SC group to 0.150 ± 0.002 g/neonate (DF = 12, $t = -1.798$, $P = 0.2919$) in HG group, and a reduction from 0.203 ± 0.002 g/neonate in the SC group to 0.188 ± 0.001 g/neonate in the HG group at P21 (DF = 13, $t = -4.435$, $P = 0.0021$). The percent reduction in cerebellar size was greatest at P6 (25.6%), the reduction (7.8%) was less pronounced at P21, and was not significant at P15. When the cerebellar size was adjusted to brain mass (grams of cerebellar tissue per gram of brain mass per neonate), the relative reduction at P6 was 11.59% (HG = 0.253 ± 0.001, SC = 0.069 ± 0.01; DF = 26, $t = -6.435$, $P < 0.0003$) and no significant difference was observed at either P15 (HG = 0.139 ± 0.001, SC = 0.135 ± 0.002; DF = 12, $t = 1.917$, $P = 0.2382$) or at P21 (HG = 0.155 ± 0.001, SC = 0.154 ± 0.003; DF = 13, $t = 0.418$, $P = 2.0481$). The cerebellar size relative to neonatal body mass was not altered in HG neonates on P6 and increased both at P15 (HG = 0.005 ± 0.0001, SC = 0.004 ± 0.00006; DF = 12, $t = 2.909$, $P = 0.0393$) and at P21 (HG = 0.004 ± 0.00008, SC = 0.0003 ± 0.00005; DF = 13, $t = 3.619$, $P = 0.0093$).

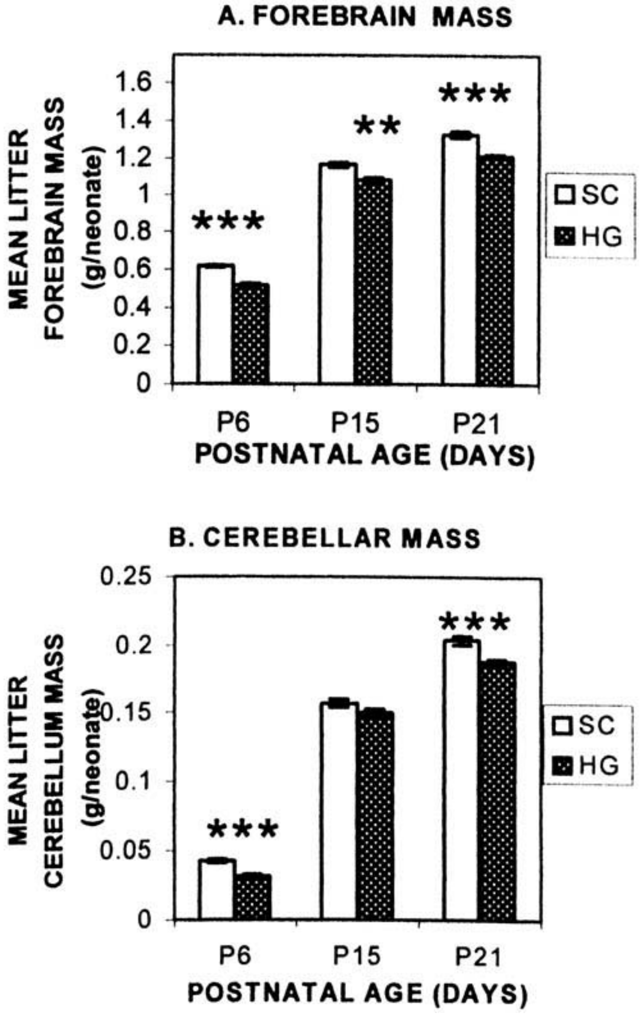

Figure 2. Effect of hypergravity on neonatal brain mass. (A) Forebrain. (B) Cerebellum. Data represent mean and SEM of litter neural tissue mass (grams per neonate). Each group contained 6 to 8 litters with 6 to 10 neonates per litter. The difference between HG and SC is indicated when significant (**$P < 0.005$, ***$P < 0.0005$).

Effect of Hypergravity on Thyroid Status. The results of TSH and total T4 and T3 analysis, performed on the dam's plasma obtained (from trunk blood) at the time of sacrifice at P6, P15, and P21, are presented in Figure 3, A through C. TSH level, the most reliable index of hypothyroidism in humans, was elevated (not significantly when Bonferroni corrected) from SC values of 4.753 ± 0.123 ng/ml to 5.986 ± 0.440 ng/ml in HG dams at P6 (DF = 12, $t = 2.352$, $P = 0.1098$) and from 5.078 ± 0.356 ng/ml in the SC group to 7.051 ± 0.535 ng/ml in the HG group at P15 (DF = 12, $t = 2.886$, $P = 0.0411$), respectively. This increase in TSH levels suggests that dams were hypothyroid. The T4 level was not altered at P6; the increased TSH without a change in free T4 level is characteristic of moderate (subclinical) hypothyroidism. At P15, an increased TSH level was accompanied by a decreased T4 level from 3.418 ± 0.170 μg/ml in SC dams to 2.837 ± 0.204 μg/ml in HG dams (DF = 12, $t = -2.083$, $P = 0.1779$), but the difference was not statistically significant when Bonferroni

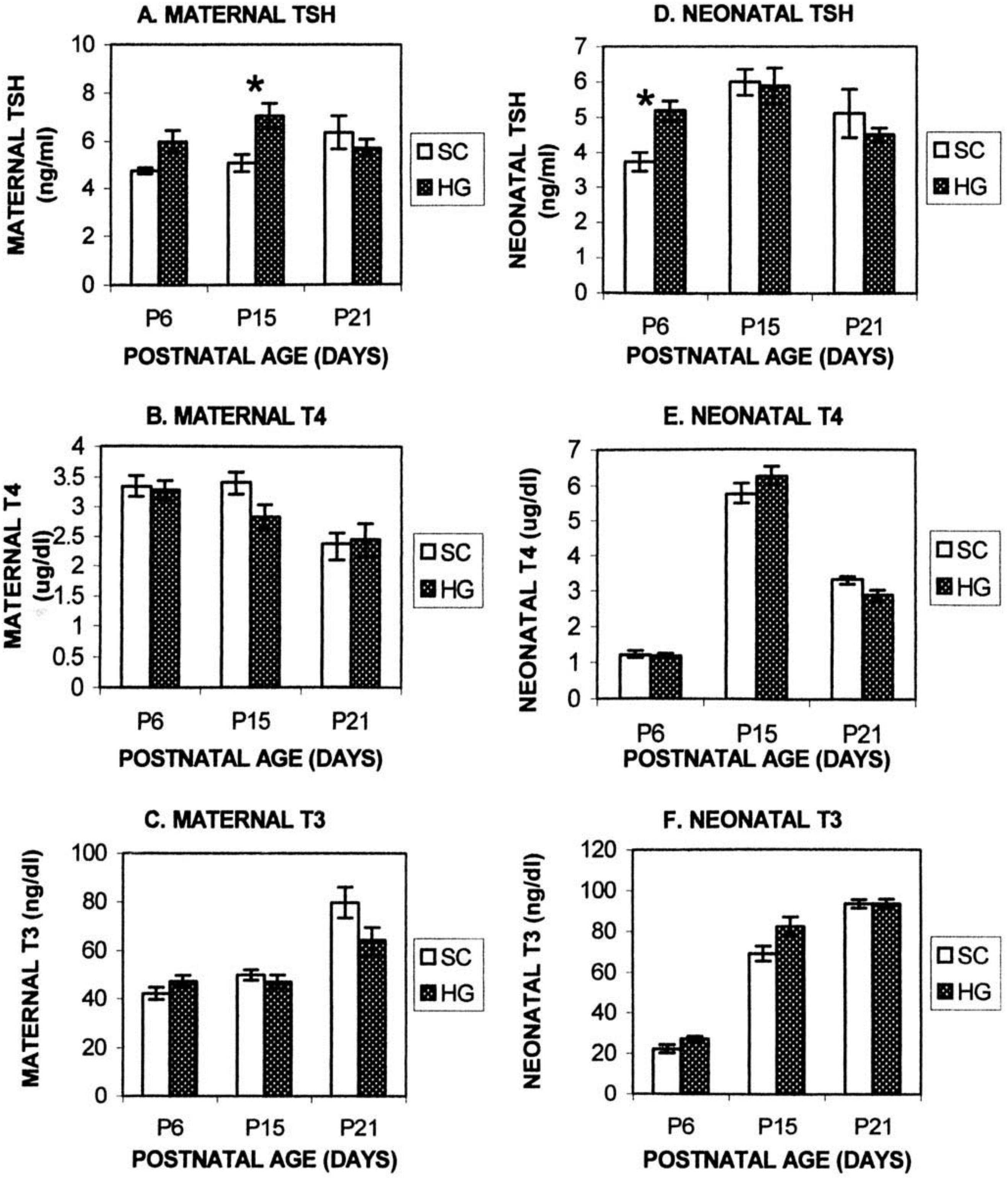

Figure 3. Effect of hypergravity on maternal and neonatal thyroid status: TSH, T4, and T3. Data represent mean and SEM of hormone concentration for each treatment group at each age. For P6 plasma analysis, the determination was based on pooled blood from two litters because of a low blood volume at this age. The difference between HG and SC values is indicated when significant (*$P < 0.05$).

corrected. The maternal T3 values were not significantly different at any time point.

Our findings indicate that until P15, the neonates' thyroid status is affected by that of the nursing dams. This relationship is further supported by a regression analysis between the maternal TSH and neonatal TSH levels ($r = 0.487$, $P = 0.0011$, Fig. 4). Elevated TSH levels in HG neonatal plasma at P6 from 3.732 ± 0.274 ng/ml in SC

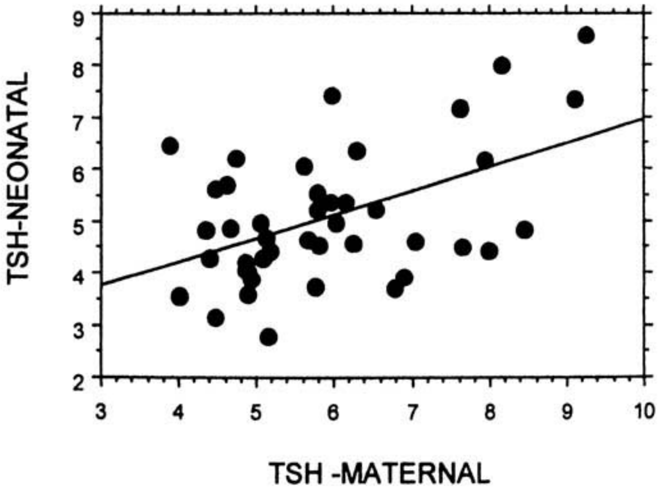

Figure 4. Relationship between neonatal and maternal TSH levels. Regression analysis indicates a significant correlation between neonatal and maternal TSH levels; $r = 0.0487$, $P < 0.001$.

neonates to 5.19 ± 0.274 ng/ml in HG neonates (DF = 12, $t = 3.599$, $P = 0.011$) suggests that the neonates exposed to hypergravity are hypothyroid (Fig. 3D). Although a small reduction in plasma total T4 levels from 1.418 ± 0.184 µg/ml in SC neonates to 1.262 ± 0.060 µg/ml in HG neonates could be observed at this time, the difference between the HG and SC neonates was not statistically significant. Thus, like their mothers, neonates are moderately hypothyroid.

A secondary reduction in plasma T4 level from 3.355 ± 0.093 µg/ml in SC neonates to 2.89 ± 0.144 µg/ml in HG neonates was observed at P21 (DF = 13, $t = -2.482$, $P = 0.0825$). No significant difference in plasma T3 level could be observed during the course of the experiment. The interpretation of these results is confounded by the dramatic developmental fluctuations in T3 and T4 levels during the early postnatal period. Thus, the difference in plasma thyroid profiles between HG and SC neonates, which has not been characterized to date, may be of importance. Such a difference is suggested by a two-way (age and gravitational condition) ANOVA with data normalized by log transformation, TSH ($F = 4.335$; $P = 0.0609$) and T4 ($F = 4.453$; $P = 0.055$).

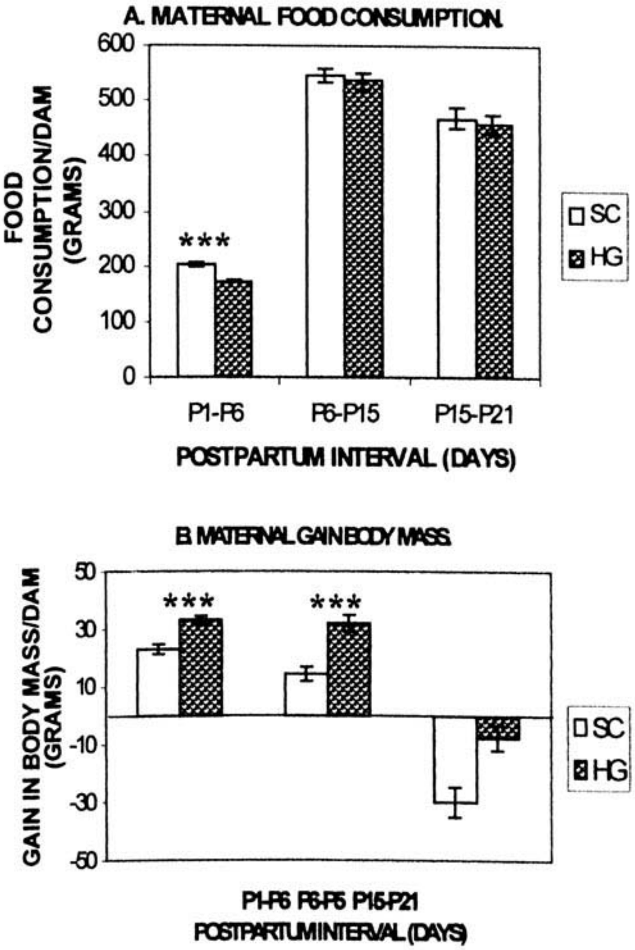

Figure 5. Effect of hypergravity on maternal food intake and gain in body mass. (A) Food consumption (grams per dam). (B) Gain in body mass (grams per dam). Data represent mean and SEM. Each group contained 6 to 8 dams. The difference between HG and SC values is indicated when significant (***$P < 0.0003$).

Effect of Hypergravity on Maternal Food Consumption and Body Weight. During the postpartum period, HG dams continued to consume less food, but the difference was significant only during the first neonatal week (P1 to P6). Food consumption (Fig. 5A) was significantly reduced during the first postpartum week (HG = 171.7 ± 4.1 g, SC = 203.1 ± 3.26 g; DF = 53, $t = -5.751$, $P < 0.0004$), but was not affected during the second postpartum week (HG = 536.3 ± 13.83 g, SC = 547.2 ± 12.08 g; DF = 26, $t = -0.585$, $P = 2.268$) or the third postpartum week (HG = 457.7 ± 16.51 g, SC = 469.5 ± 17.21 g; DF= 13, $t = -0.496$, $P = 2.716$). The relative food consumption adjusted to body mass was, however, not affected during the first (HG = 0.701 ± 0.02 g/g body wt, SC = 0.712 ± 0.014 g/g body wt; DF = 53, $t = -0.406$, $P = 2.7452$) or the second neonatal week (HG = 1.94 ± 0.06 g/g body wt, SC = 1.77 ± 0.044 g/g body wt; DF = 26, $t = 2.244$, $P = 0.13344$.

During the first two postpartum weeks, HG dams gained more weight (Fig. 5B), both in absolute and relative terms (adjusted to body mass), than did the SC dams. Gain in maternal body mass at the end of each postpartum week (1st: P1 to P6; 2nd: P6 to P15, 3rd: P15 to P21) is shown in Figure 5B. During the first postpartum week, HG dams gained 42.4% more weight than did the SC dams (HG = 33.3 ± 1.53 g, SC = 23.39 ± 1.74 g; DF = 56, $t = 4.27$, $P < 0.0004$) and during the second postpartum week, HG dams gained 120.5% more weight than did SC dams (HG = 32.2 ± 2.81 g, SC = 14.59 ± 2.42, DF = 28, $t = 4.568$, $P < 0.0004$). During the third postpartum week, both HG and SC dams lost body mass (HG = 7.7 ± 4.29, SC = 62 ± 32.34; DF = 14, $t = 1.663$, $P = 0.4744$), but the difference was not significant. The relative gain in body weight at the end of the first postpartum week was increased by 65% in

HG dams (HG = 0.136 ± 0.007, SC = 0.082 ± 0.007; DF = 56, t = 5.65, $P < 0.0004$), and at the end of second week, increased by 146.8% (HG = 0.116 ± 0.010 g/g body wt, SC = 0.047 ± 0.008 g/g body wt; DF = 28, t = 5.134, $P < 0.0004$). By the end of the third postpartum week, the HG dams lost less weight (−0.025 ± 0.014 g/g body wt) than the SC dams (−0.204 ± 0.114 g/g body wt), but the difference was not significant (DF = 14, t = 1.562, P = 0.562).

Discussion

Results presented in this study indicate that exposure to hypergravity (1.5 G) during gestation and the early postnatal period affects neonatal growth, the developing CNS, and the thyroid status of rat neonates.

Hypergravity-exposed neonates weighed less, and both their brain size and cerebellar size were decreased. The reduction in cerebellar size at P6 was greater than in the forebrain, as suggested by both absolute decrease in mass (cerebellum, 25.6% decrease; forebrain, 15.9% decrease) and the decrease in relative cerebellar mass (adjusted to forebrain mass), suggesting that the cerebellum may be more sensitive to hypergravity during this period corresponding to the most active cerebellar development. The use of any of the relative bases for evaluating changes in organ mass, such as a percentage of body weight or fat-free body weight (FFBW), has been suggested for adult animals (24) in which growth is not a contributing factor. Similarly, the CNS mass has been used as a weight-stable organ and as a reference for changes in mature organs (24). In the case of the developing CNS, the use of such relative values may not be appropriate. Brain mass is not stable at this time, and the brain growth reflects the increase in the number of neuronal cells, fibers, and myelin, whereas general body growth is induced as a result of an increase in a number of different types of cells (i.e., muscle, fat, and bone), extracellular matrix, and cellular volume. Furthermore, general body and neuronal growth are regulated by different mechanisms, and growth hormone does not play a major role in neuronal growth. For these reasons, we have used absolute-mass values rather than relative values for the comparison between SC and HG animals.

When adjusted to body mass, there was a 10.8% increase in forebrain mass at P6 and no significant change at P15 or at P21. An increase in the ratio of forebrain to body mass at P6 suggests that at this time, the hypergravity may exert a brain-sparing effect. On the other hand, when the cerebellar results are expressed relative to body mass, the ratio is similar in HG and SC neonates, suggesting that the hypergravity, unlike undernutrition, does not result in "cerebellum-sparing" effect. Furthermore, when the cerebellar mass was adjusted to forebrain mass, the overall effect of hypergravity on cerebellum size at P6 persisted even if the magnitude of change was less pronounced, further supporting the claim that during neonatal development, the cerebellum is more sensitive to hypergravity exposure than is the rest of the brain.

Although a few studies have explored the effect of altered gravity on the developing CNS, none of them has dealt systematically with changes in the developing CNS. In the present study, the hypergravity paradigm was selected because it can best accommodate the requirements of the developmental studies to include a large number of pregnant and lactating dams, births, and nursing offspring. Many organs and functions affected by exposure to hypergravity are also known to be sensitive to microgravity, although some show changes in the opposite direction. Thus, it is important to stress that changes in both the thyroid status and the CNS in response to micro- and hypergravity appear to be similar. In comparing the results of altered gravity on the CNS in developing animals, several factors, such as the duration of exposure and the phase of development, as well as the CNS region, are of importance. Most of the microgravity studies, limited to the prenatal period, showed neuronal degeneration in various brain regions, retarded synaptogenesis in the vestibular nuclei (25, 26), and altered morphology of cortex and cerebellum and vestibular system (27–29), but the effects of exposure to microgravity were transient (29, 30). Studies of the functional development of rats exposed to microgravity toward the end of gestation (5 days) showed no abnormalities (31), but a longer exposure to microgravity (9 to 11 days) during that period resulted in changes of some vestibular functions (32). On the other hand, exposure to hypergravity (1.8 G) for 26 days from G11 to P15 (33) resulted in a substantial delay in monoaminergic projections to the spinal cord. In the present study, exposures to hypergravity ranging in duration from 17 to 32 days and encompassing the second part of embryonic and the entire neonatal development to weaning covered the critical period of cerebellar development. It also coincides with the period of maturation of posture and locomotion (34).

The changes in the neonatal developing CNS observed in the present study are accompanied by altered thyroid status of both neonates and dams. The increase in plasma TSH level, the most reliable index of thyroid status in humans (35–37) and in rodents (48), is consistent with a hypothyroid state in the HG neonate at the end of the first week, a time of maximal difference in cerebellar size. The fact that plasma TSH elevations are not accompanied by T4 changes indicates a moderate hypothyroidism (39). However, even moderate hypothyroidism in dams, characterized by increased plasma TSH but normal T4, has a negative effect on the availability of T4 to fetuses (14). It is thus possible that the subnormal TH levels reaching the developing CNS in the hypergravity-exposed neonates contribute to the observed reduction in the size of the brain and, specifically, the cerebellum. Although dams can mitigate TH deficiency in the fetal brain (40), the maternal TSH levels indicate that dams are also hypothyroid at this time, in agreement with earlier findings (5). Moderate hypothyroidism in pregnant women is, however, sufficient to affect the neurological development of human fetuses (44).

In the neonate, the pituitary-thyroid axis and TH levels are established during the early neonatal period to accommodate rapid growth and development (42). Previous results reported for control Sprague-Dawley rats (43) indicate that neonatal plasma T4 levels, low at birth, rise by the end of the second postnatal week, and that plasma T3 levels double during the first month of life. Our results are in general consistent with these findings. Changes in both T4 and T3 during the neonatal period suggest that in addition to point-by-point comparisons, the alteration in the TH developmental profile is relevant in evaluating the effect of hypergravity on the neonate's thyroid status. Analysis of the time course of T3 and T4 suggests changes in TH developmental periodicity in HG neonates. Low levels of plasma TH during the first postnatal week limited the previous (43) and present analyses to total T4 and T3. In the developing CNS, it is the circulating free T4 level that contributes to the T3 found within brain cells (44). During pregnancy, the maternal T4 is transported to the fetus and is crucial to fetal CNS development (45). The results of ANOVA suggest that the developmental profile of plasma TH is altered in hypergravity-exposed neonates.

The effect of hypergravity on neonates is especially pronounced at P6. The neonates, already of smaller size at birth, remain smaller than the controls, as evidenced by a persistently low body weight. Forebrain and especially cerebellum size are decreased maximally at P6, and these changes coincide with the hypothyroid state of the neonate, thereby supporting our hypothesis of TH involvement in the mediation of the effect of hypergravity on the CNS.

It is also possible, however, that undernutrition may contribute to the effects of hypergravity observed in the developing neonates. Nutrition during early development affects growth in general, including that of the CNS (46). Data on food consumption and gain in body weight suggest that during pregnancy, the hypergravity-exposed dams consumed less food and gained less weight. It is thus possible that during pregnancy, undernutrition may contribute to the lower body mass of HG neonates. However, two points argue against the direct contribution of maternal undernutrition to the effect of hypergravity on the developing neonates. First, during lactation, despite lower food consumption during the first postpartum week and similarly low food consumption during the second postpartum week, HG dams gained significantly more body mass (and lost less mass during the third postpartum week) than did SC dams. Furthermore, when the neonatal values of cerebellar size are adjusted to either maternal food consumption or body mass, hypergravity-exposed neonates still show a significant difference from SC neonates. Second, in undernourished rats, TSH is usually lower because of a disrupted pituitary axis; in both fasting adults (47, 48) and newborn (49) rats, plasma TSH and TH were decreased. However, in the present study, TSH was actually increased in the HG neonatal plasma (Fig. 3C). Thus, while the maternal nutritional status is not affected, the transient neonatal hypothyroidism coincides with maximal changes in the cerebellum, lending support to our hypothesis that TH is involved in mediating the effect of hypergravity on the CNS.

From the preceding discussion, it is apparent that the mechanism(s) involved in hypergravity-associated changes in CNS are complex, but the role of TH in hypergravity effects on the developing CNS merits serious consideration. The developing rat cerebellum, which shares many characteristics of human brain during the last trimester of pregnancy and the first several months of life, has been used extensively as a model for the study of the effect of various environmental factors on the CNS. The data presented here suggest that the developing rat cerebellum may also be considered a good model for predicting changes in the developing human CNS under altered gravity. The present study begins to define the physiological mechanisms involved in the overall response of the developing organism to altered gravity. Future studies of rat cerebellar development under hypergravity are critical in developing an understanding of how altered gravity in space may affect human brain development, and they may help to predict the feasibility of long-term human survival in space.

We express our gratitude to the NASA/ARC Life Sciences Engineering Branch, and specifically to Jim Connolly and Tianna Shaw for arranging access to the Hypergravity Facility and for their support during the experiments. We thank Megan Moran, Tom Wang, Fang Yuan, Nicole Mills, and Kieu Lam for assistance with data collection. We especially thank Mari Miller and Mary Eyestone for assistance with hormone analysis. We thank Jon Griffith, Dan Gundo, and Tony Purcell for 24-ft centrifuge set-up and support and Dr. Carolyn Reed, David Garcia, and Mary Williams for veterinary and animal care. We acknowledge Dr. Nancy Dauton for helpful advice in experimental planning. We also thank Jaylyn Olivo and Nancy Voynow of Brigham and Women's Hospital for excellent editorial assistance.

1. Strollo F. Hormonal changes in humans during spaceflight. Adv Space Biol Med **7**:99–129, 1999.
2. Grindeland RE, Popova IA, Vasques M, Arnaud SB. Cosmos 1887 mission overview: Effects of microgravity on rat body and adrenal weights and plasma constituents. FASEB J **4**:105–109, 1990.
3. Merrill A Jr, Wang HE, Mullins RE, Grindeland RE, Popova IA. Analyses of plasma for metabolic and hormonal changes in rats flown aboard COSMOS 2044. J Appl Physiol **73**:s132–s115, 1992.
4. Plakhuta-Plakutina, GI, Kabitskii EN, Dmitrieva NP, Amirkhanian EA. Studies of the morphology of the thyroid gland and thyroid hormone levels in the blood of rats in experiments on "Kosmos-1667" and "Kosmos-1887". Kosm Biol Aviakosm Med **24**:25–27, 1990.
5. Megory E, Oyama J. Hypergravity effects on liter size, nursing activity, prolactin, TSH, T3 and T4 in the rat. Aviat Space Environ Med **55**:1129–1135, 1984.
6. Newberg AB. Changes in the central nervous system and their clinical correlates during long-term spaceflight. Aviat Space Environ Med **65**:562–572, 1994.
7. Katafuchi T, Hori T, Oomura Y, Koizumi K. Cerebellar afferents to neuroendocrine cells: Implications for adaptive responses to simulated weightlessness. Endocr J **42**:729–737, 1995.
8. Serova LV. Hypergravity and development of mammals. Physiologist **34**:s135–s136, 1991
9. Fox N, Damjanov AI, Martinez-Hernandez A, Knowles BB, Solter D. Immunohistochemical localization of early embryonic antigen (SSEA-

1) in post-implantation mouse embryos and fetal and adult tissues. Dev Biol **83:** 391–398, 1981.

10. Sondag HN, de Jong HA, Oosterveld WJ. Altered behavior in hamsters conceived and born in hypergravity. Brain Res Bull **43:**289–294,1997.
11. Nicholson JL, Altman J. The effect of early hypo- and hyperthyroidism on the development of rat cerebellar cortex. I. Cell proliferation and differentiation. Brain Res **44:**13–23, 1972.
12. Oppenheimer JH, Schwartz HL. Molecular basis of thyroid hormone-dependent brain development. Endocr Rev **18:**462–475, 1997.
13. Obregon MJ, Santisteban P, Rodriguez-Pena A, Pascual A, Cartagena P, Ruiz-Marcos A, Lamas L, Escobar del Rey F, Morreale de Escobar G. Cerebral hypothyroidism in rats with adult-onset iodine deficiency. Endocrinology **115:**614–624, 1984.
14. Morreale de Escobar G., Obregon MJ, Ruiz de Ona C, Escobar del Rey F. Transfer of thyroxine from the mother to the rat fetus near term: effects on brain 3,5,3′-triiodothyronine deficiency. Endocrinology **122:**1521–1531, 1988.
15. DeLong GR, Stanbury JB, Fierro-Benitez R. Neurological signs in congenital iodine-deficiency disorder (endemic cretinism). Dev Med Child Neurol **27:**317–324, 1985.
16. Porterfield SP, Hendirch CE. The role of thyroid hormones in prenatal and neonatal neurological development-current perspectives. Endocr Rev **14:**94–106, 1993.
17. Altman J. Morphological and behavioral markers of environmentally induced retardation of brain development: An animal study. Environ Health Perspect **74:**153–168, 1987.
18. Legrand J. Morphogenetic actions of thyroid hormones. Trends Neurosci **2:**234–236, 1979.
19. Legrand J. Thyroid hormone effects growth and development. In: Henneman G, Ed. Thyroid Hormone Metabolism. New York: Marcel Dekker, pp503–534, 1986.
20. Sajdel-Sulkowska EM, Koibuchi N. Altered CD15 glycolipid expression in developing rat cerebellum following treatment with antithyroid drug propylthiouracil. Endocr J **47:**353–358, 2000.
21. Versloot PM, van der Heide D, Schroder-van der Elst JP, Boogerd L. Maternal thyroxine 3,5,3′-tri-iodothyronine kinetics in near-term pregnant rats at two different levels of hypothyroidism. Euro J Endocr **138:**113–119, 1998.
22. Patel AJ, Rabie A, Lewis PD, Balazs R. Effect of thyroid deficiency on postnatal cell formation. Brain Res **104:**33–46, 1976.
23. Oyama J, Solgaard L, Corrates J, Monson CB. Growth and development of rats conceived and reared at different G intensities during chronic centrifugation. Physiologist **28:**583–584, 1985.
24. Pitts GC, Bull LS, Oyama J. Effects of chronic centrifugation on body composition in the rat. Am J Physiol **223:**1044–1048, 1972.
25. Bruce LL, Fritzsch B. The development of vestibular connections in rat embryos in microgravity. J Grav Physiol **4:**59–62, 1997.
26. Savel'ev SV, Serova LV, Besova NV, Nosovskii AM. Effect of weightlessness on rats endocrine system development. Aviakosm Ekolog Med **32:**31–36, 1998.
27. Krasnov IB, Olenev SN, Babichenko II. Morphogenesis of the brain of rats developing under conditions of weightlessness. Kosm Biol Aviakosm Med **21:**16–22, 1987.
28. Krasnov IB, Dyachkova LN. The effect of space flight on the ultrastructure of the rat cerebellar and hemisphere cortex. Physiologist **33:**s29–s30, 1990.
29. Keefe JR, Alberts JR, Krasnov IB, Serova LV. Developmental morphology of the eye, vestibular system and brain in 18 day fetal and newborn rats exposed *in utero* to null gravity during the flight of Cosmos 1514. NASA Technical Memorandum **88223**, 1986.
30. Malacinski GM, Neff AW, Alberts JR, Souza KA. Developmental biology in outer space. BioScience **39:**314–320, 1989.
31. Alberts JR, Serova LV, Keefe JR, Apanasenko Z. Early postnatal development of rats gestated during flight of Cosmos 1514. Physiologist **28:**s81–s82, 1986.
32. Ronca AE, Alberts JR. Altered vestibular function in fetal and newborn rats gestated in space. J Grav Physiol **4:**63–66, 1997.
33. Gimenez y Ribotta M, Sandillon F, Privat A. Influence of hypergravity on the development of monoaminergic systems in the rat spinal cord. Brain Res Dev Brain Res **111:**147–157,1998.
34. Clarac F, Vinayl L, Cazalets JR, Fady JC, Jamon M. Role of gravity in the development of posture and locomotion in the neonatal rat. Brain Res Brain Res Rev **28:**35–43, 1998.
35. Tatsumi K, Takeoka KT. Amino N. Serum TSH measurements. Nippon Rinsho **57:**1806–1809, 1999.
36. Hallengreen B. Hypothyroidism: Clinical findings, diagnosis, therapy. Thyroid tests should be performed on broad indications. Lakartianingen **16:**4091–4096, 1998.
37. Rotti E, Gardini E, Magotti MG, Pilla S, Minelli R, Salli M, Monica C, Maestri D, Cencetti S, Braverman LE. Are thyroid function tests too frequently and inappropriately requested? J Endocrinol Invest **22:**184–190, 1999.
38. Colzani R, Fang SL, Alex S, Braverman LE. The effect of nicotine on thyroid function in rats. Metabolism **47:**154–157, 1998.
39. Evered DC, Ormston BJ, Smith PA, Hall R, Bird T. Grades of hypothyroidism. Br Med J **1:**657–662, 1973.
40. Morrelale de Escobar G. Maternal-fetal thyroid relationships and the fetal brain. Acta Med Austriaca **15**(Suppl 1):66–70, 1988.
41. Smit BJ, Kok JH, Vulsma T, Briët JM, Boer K, Wiersinga WM. Neurological development of the newborn and young child in relation to maternal thyroid function. Acta Paediatr **89:** 291–295, 2000.
42. Gayo L, Bonet B, Herranz AS, Iglesias R, Toro MJ, Montoya E. Postnatal development of brain TRH, serum TSH and thyroid hormones in the male and female rat. Acta Endocrinol **112:**7–11, 1986.
43. Pracyk JB, Seidler FJ, McCook EC, Slotkin TA. Pituitary-thyroid axis reactivity to hyper- and hypothyroidism in the perinatal period: Ontogeny of regulation of regulation and long-term programming of responses. J Dev Physiol **18:**105–109, 1992.
44. Robbins J, Lakshmanan M. The movement of thyroid hormones in the central nervous system. Acta. Med Austriaca **19**(Suppl 1):21–25, 1992.
45. Sinha AK, Pickard MR, Ekins RP. Maternal hypothyroxinemia and brain development: A hypothetical control system governing fetal exposure to maternal thyroid hormones. Acta Med Austriaca **19**(Suppl 1):40–48, 1992.
46. Gopinath G, Bijlani V, Deo MG. Undernutrition and the developing cerebellar cortex in the rat. J Neuropathol Exp Neurol **32:**125–135, 1976.
47. Croxson MS, Hall TD, Kletzky OA, Jaramillo JE, Nicoloff JT. Decreased serum thyrotropin induced by fasting. J Clin Endocrinol Metab **45:**560–568, 1977.
48. Harris ARC, Fang S-H, Azizi F, Lipworth L, Vagnakis AG, Braverman LE. Effect of starvation on hypothalamic-pituitary-thyroid function in the rat. Metabolism **27:**1074–1083, 1978.
49. Aláez C, Calvo R, Obregón MJ, Pascual-Leone AM. Thyroid hormone and 5′-deiodinase activity in neonatal undernourished rats. Endocrinol **130:**773–779, 1992.