

Maternal Reproductive Experience Enhances Early Postnatal Outcome Following Gestation and Birth of Rats in Hypergravity¹

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ABSTRACT

A major goal of space life sciences research is to broaden scientific knowledge of the influence of gravity on living systems. Recent spaceflight and centrifugation studies demonstrate that reproduction and ontogenesis in mammals are amenable to study under gravitational conditions that deviate considerably from those typically experienced on Earth ($1 \times g$). In the present study, we tested the hypothesis that maternal reproductive experience determines neonatal outcome following gestation and birth under increased (hyper) gravity. Primigravid and bigravid female rats and their offspring were exposed to $1.5 \times g$ centrifugation from Gestational Day 11 either through birth or through the first postnatal week. On the day of birth, litter sizes were identical across gravity and parity conditions, although significantly fewer live neonates were observed among hypergravity-reared litters born to primigravid dams than among those born to bigravid dams (82% and 94%, respectively; $1.0 \times g$ controls, 99%). Within the hypergravity groups, neonatal mortality was comparable across parity conditions from Postnatal Day 1 through Day 7, at which time litter sizes stabilized. Maternal reproductive experience ameliorated neonatal losses during the first 24 h after birth but not on subsequent days, and neonatal mortality was associated with changes in maternal care patterns. These results indicate that repeated maternal reproductive experience affords protection against neonatal losses during exposure to increased gravity. Differential mortality of neonates born to primigravid versus bigravid dams denotes gravitational load as one environmental mechanism enabling the expression of parity-related variations in birth outcome.

parturition, pregnancy, reproductive behavior, stress

INTRODUCTION

The transition from the first to the second pregnancy and lactation is accompanied by characteristic neural, endocrine, and behavioral changes in mothers. Reduced circulating levels of prolactin [1], increased prolactin response to neonates in the absence of suckling [2], diminished opioid content of the medial preoptic area [3], decreased sensitivity to disruptive effects of morphine on retrieving of pups [4], reductions in neural sensitivity to opioids coupled with increased maternal care [1], and neuroanatomical changes [5, 6] are all associated with prior parity. Maternally experienced rats are less vulnerable than naïve (pri-

miparous) ones to the disruptive effects of various endocrine [7–9] and sensory or neural [10, 11] manipulations. As impressive as these data are in the aggregate, clear parity-related shifts in patterns of maternal care have been relatively difficult to discern in observational studies [12–15]. In other studies, utilizing more detailed and sensitive tests, improvements in retrieving behavior have been reported across parities in rats [16, 17]. However, none of these investigations indicate that prior parity affects neonatal survival or well-being. The clearest evidence for parity-related effects on infants comes from studies in which mothers have been exposed to unusual or challenging environmental conditions. For example, in ungulates, disruption of either neonatal cues [18] or maternal-neonatal interactions at birth [19, 20] are associated with higher rates of neonate rejection by primiparous than by multiparous mothers. Thus, the common belief that reproductively experienced mothers are “better” mothers [21] may hold true under some circumstances.

The maternal care system in mammals evolved within the confines of the Earth's $1 \times g$ gravitational field. It is therefore not surprising that when gravity is altered corresponding changes are observed in mothers and offspring [22, 23]. Spaceflight and centrifugation, unique tools for increasing or decreasing gravitational load relative to that typically experienced on Earth ($1 \times g$), have been used by some investigators to study gravity's effects on reproducing and developing mammals. The studies conducted to date indicate that the period spanning mid- to late pregnancy is not interrupted by exposure to spaceflight [24, 25]. In contrast, significant mortality was reported among postnatal rats flown in space [23]. Neonatal deaths may have been caused by the mother's inability to keep the weightless, free-floating pups gathered so that adequate huddling and nursing could take place. Without the warmth and sustenance that the mother normally provides, altricial neonatal rats are likely to become hypothermic and starved, leading to rapid physiological decline. Similar neonatal outcomes have been reported at the opposite end of the gravity continuum. Under conditions of increased gravity (hypergravity; either $2.16 \times g$ or $3.14 \times g$), rat mothers that gave birth during centrifugation were reported to be less “maternal,” and neonatal survival was diminished relative to $1 \times g$ controls [26]. In both hypo- and hypergravity, disruptions of mother-infant interactions have been implicated in the deaths of neonates. As gravitational load changes, so does the physical relationship between mothers and their pups. For example, the weightless space environment and the weighted hypergravity environment could each hinder the mother's ability to assume kyphosis [27], a characteristic upright, arched-back nursing posture. Exposure to altered gravity may also impair the mother's ability to maneuver infants, thereby limiting characteristic licking and nursing. Infants, in turn, must be sufficiently healthy and strong to attach to a nipple and suckle under atypical grav-

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itational loads. Sufficient numbers of pups must be present at the nipple to induce quiescence and the kyphosis posture in the mother [27]. Fluid dynamics, core temperature, muscle mass, calcium metabolism, and bone density also change during exposure to increased or decreased gravitational loads [28, 29] and are likely to alter the physiological functions of both mothers and infants. Specific maternal processes related to nursing and lactation, such as mammary metabolism, are also affected [30, 31]. Alterations in maternal hormonal status [26] or changes in sensory characteristics of the newborns [18, 32] may impede the onset and maintenance of maternal care. These findings suggest that studies of reproducing and developing animals in altered gravity environments will be useful for advancing our understanding of how gravitational forces have shaped these fundamental aspects of mammalian life.

In the present study, we examined the role of maternal factors in litter outcomes under modest ($1.5 \times g$) hypergravity conditions. We hypothesized that when dams are exposed to hypergravity during pregnancy, parturition, and lactation, the survival and well-being of their offspring is enhanced if mothers are reproductively experienced (bigravid) rather than inexperienced (primigravid). We predicted that the most pronounced parity differences would be observed during the period immediately following birth, corresponding to the time of maximal maternal responsiveness toward the young [33]. To test our hypotheses, primi- and bigravid rat dams were exposed to continuous $1.5 \times g$ centrifugation from Gestational Day (G) 11 of the 22-day pregnancy throughout birth, with some of the litters remaining on the centrifuge throughout the first postnatal week. To quantify the influence of maternal parity on neonatal outcome in hypergravity, we recorded at birth 1) the total number of progeny, 2) the number of live progeny, 3) dam and pup body mass, and 4) gender of the offspring. During the first postnatal week, litter sizes and body weights were recorded. Behavioral data were derived from time-lapse video recordings made onboard the centrifuge. This is the first study of maternal parity in mammals developing under altered gravity. The comparisons described herein are important for understanding the effects of repeated reproductive experience on the maternal care patterns and survival of young reared in altered gravitational fields. Our observations yield novel insights into the role of environmental factors in the expression of parity-related variations in maternal care.

MATERIALS AND METHODS

Subjects

Animal experimentation was conducted in accordance with the guidelines of the NASA Institutional Animal Care and Use Committee and the National Research Council Guide for the Care and Use of Laboratory Animals. Time-bred female Sprague-Dawley rats ($n = 110$) weighing between 190 and 290 g, were shipped from the vendor (Taconic Farms, Germantown, NY) to the NASA Ames Research Center (ARC, Moffett Field, CA) on G2 (the day spermatozoa were observed in a vaginal lavage = G1). Primigravid ($n = 52$) and bigravid ($n = 36$) dams were used in the study. Bigravid dams underwent their first pregnancy and lactation at the vendor's facility prior to rebreeding and shipment. It was not feasible to precisely age-match dams from the two different parity conditions. Bigravid dams were approximately 2–4 wk older than were primigravid dams (11–12 wk versus 8–9 wk). Throughout the experiment, dams were individually housed in maternity cages ($47 \times 26 \times 21$ cm) lined with corn cob bedding and maintained under standard colony conditions (12L:12D cycle [lights-on: 0600 h], $21^\circ\text{C} \pm 1^\circ\text{C}$, 30–50% humidity). Rat chow (Purina 5102, Richmond, IN) and water were available ad libitum.

Apparatus

Hypergravity ($1.5 \times g$) was produced using the NASA ARC 24-foot-diameter centrifuge, which consists of a central vertical shaft spindle driven by a 25-horsepower motor. Attached to the top of the spindle, approximately 6 feet above the floor, are 10 radial arms. Each arm holds an enclosure ($9.3 \times 15.6 \times 6.7$ cm), which is gimbaled during operation such that the hypergravitational field is applied through the floor of the cage. Ten enclosures, each capable of housing four maternity cages, were positioned 12 feet (3.60 m) from the center axis. Rotation rate was set at 15.94 RPM, thereby augmenting the earth's gravitational force ($1.0 \times g$) by 50% to produce an overall load equal to $1.5 \times g$ to the dams' cages. Two additional enclosures positioned 6 feet (1.80 m) from the center axis were subject to $1.08 \times g$ during centrifugation to control for coriolis (i.e., rotational) effects.

Treatment Groups

Following arrival at the ARC, each dam was weighed daily from G2 until G9 to confirm pregnancy status. On G9, a total of 88 dams (52 primigravid and 36 bigravid) were selected for inclusion in the study based upon daily body weight gain during the preceding week. Each dam was assigned to one of four experimental conditions, with body weights carefully matched across groups.

Hypergravity. Primigravid ($n = 16$) and bigravid ($n = 16$) dams were placed in outer radius centrifuge enclosures and exposed to $1.5 \times g$. The average body mass (\pm SD) in the hypergravity (HG) group at the start of centrifugation on G11 was 257.5 ± 7.7 g for primigravid dams and 309.0 ± 19.5 g for bigravid dams.

Stationary yoked control. Primigravid ($n = 16$) and bigravid ($n = 16$) dams were housed throughout the experiment in a separate colony room. Environmental conditions and daily handling were matched to the HG group except for centrifugation. These stationary yoked control (SYC) dams were yoked one-to-one with HG dams according to body weight. Following parturition, each SYC litter was adjusted daily in size and gender composition to match the yoked HG litter if mortality occurred in the HG group. The average body mass (\pm SD) in the SYC group on G11 was 252.1 ± 11.4 g for primigravid dams and 302.4 ± 22.6 g for bigravid dams.

Rotational control. Primigravid ($n = 4$) and bigravid ($n = 4$) dams were placed within inner radius centrifuge enclosures and exposed to $1.08 \times g$. The average body mass (\pm SD) in the rotational control (RC) group on G11 was 258.8 ± 12.0 g for primigravid dams and 306.8 ± 29.2 g for bigravid dams.

Vivarium control. Only primigravid ($n = 16$) dams were assigned to the vivarium control (VIV) condition. Throughout the experiment, these dams were housed in a separate colony room under conditions matching those of the HG dams except for centrifugation. Dams and litters remained undisturbed after birth except for daily counting of neonates. The average body mass (\pm SD) for VIV dams on G11 was 256.2 ± 11.3 g.

Treatment Protocol

On G9, HG and RC dams were loaded onto the 24-foot centrifuge with either two or four maternity cages per centrifuge enclosure. Centrifugation was initiated on G11 and continued either throughout birth (Postnatal Day [P] 0, $n = 34$ litters) or throughout the first postnatal week (P7, $n = 50$ litters). The centrifuge stopped daily at 0800 h (for 30–60 min) for veterinary checks, animal maintenance, and data collection. For each litter, the first day that neonates were observed within the nest during the daily stop was designated as P0. Total litter size, the number of live and dead neonates, gender, and body weights were recorded. Eight neonates (four males and four females) were randomly selected from each litter, individual identifications were assigned by applying colored ink to the dorsum with permanent felt-tip markers, and newly assembled litters were returned to the original dam. Excess neonates were killed with CO_2 . Each mother retained her own offspring after birth; we did not attempt to control for postnatal maternal effects via cross-fostering procedures. We anticipated that hypergravity exposure would increase neonatal mortality, thereby leading to smaller overall litter sizes under the HG conditions. In polytocuous species, milk yield and body weight gain per pup are inversely related to litter size [34]. It was therefore important to control for neonatal losses within the HG group by matching litter sizes across the HG and SYC conditions. We did this by noting on a daily basis the number of dead or missing neonates in HG litters and then removing the equivalent number of offspring from the corresponding SYC litter. Gender composition of the matched HG litter was preserved in daily SYC litter adjust-

ments. A nonmanipulated VIV group was maintained as a control for shrinking litter size.

Videographic Observations

During parturition (on either G22 or G23), primigravid and bigravid HG and SYC dams ($n = 4$ per condition) were videotaped in a time-lapse manner (6:1 record:playback ratio). We quantified characteristic behaviors of the dams during parturition: 1) licking and handling of each pup, 2) dam-pup contact and 3) placentophagia. During the 3 h following parturition, we quantified pup retrieval and grouping, nursing, litter distribution within and outside of the nest, and the mother's activities within the nest. Beginning on the evening of P0 and until P6, dams and their litters were videotaped for 24 h at a slower tape speed (12:1 record:playback ratio). Red lighting was used to enable visualization of the dams and litters during the dark phase of the circadian cycle. On P0, P3, and P5, 6-h samples taken during the light and dark phases of the cycle (1200–1800 h and 0000–0600 h, respectively) were analyzed by trained scorers. Tape review was conducted in real time and was time locked to a computerized event-scoring program (STAR Enterprises, Bloomington, IN). We quantified three predominant maternal behaviors in the dams: 1) pup licking, 2) retrieving, 3) nursing. We also analyzed a fourth behavior category that we termed non-pup-directed maternal behavior. Behaviors in this category consisted of tail retrieving and disrupting the bedding within the nest. For the video analyses, inter-rater reliability was very high ($R^2 > 0.98$).

Data Collection and Analysis

Following parturition, neonates were counted and the gender of each individual was noted. Analyses of pregnancy and initial postpartum outcome on P0 were composed of data from all gravid dams and their litters ($n = 88$). Analyses of postnatal survival were derived from a subset of dams and litters ($n = 50/88$) retained in the study until P7. Where appropriate, numerical data are expressed as litter averages (mean \pm SD). For statistical purposes, multiple offspring from the same dam do not represent repeated, independent observations [35]. Rather, offspring from a given dam represent only a single observation because the littermates share in common a dam, a genetic substrate, and a host of environmental variables that are not shared with offspring from another litter. Littermates are therefore considered nonindependent observations. Accordingly, data from each litter were averaged for purposes of presentation and statistical analysis. Data were analyzed using a one-way analysis of variance (ANOVA) or a t -test for analysis of simple effects. Post hoc comparisons were made using the Newman-Keuls (NK) test. Nominal data were analyzed using a chi-square analysis. Relationships among variables were analyzed using simple and multiple regression. Primigravid but not bigravid dams were assigned to the VIV condition, yielding an unbalanced experimental design. Data from the VIV condition are therefore included only in selected, relevant analyses.

RESULTS

Pregnancy Outcome

We observed that a small number of dams in the SYC and HG conditions were not pregnant (Table 1). Pregnant dams in all groups underwent parturition at the expected time (on G22 and G23 for the Norway rat). Regardless of parity, a significantly greater number of HG dams gave birth on G23 as compared with SYC and RC dams (HG: 48%, SYC: 7%, RC: 0%; HG vs. SYC: $\chi^2 [1] = 10.8$; $P < 0.001$).

Litter sizes and gender ratios were similar across gravity conditions (Table 1); however, birth weights were significantly reduced in the HG and RC litters relative to the SYC and VIV litters ($F [3,79] = 22.27$, $P < 0.0001$; NK test, $P < 0.05$). Post hoc analyses revealed that birth weights of SYC and VIV litters did not differ from one another ($P > 0.05$, NS). Although litter sizes on P0 were comparable across gravity and parity conditions, primigravid (P) HG litters were composed of significantly fewer live neonates than were SYC, RC, or VIV litters (Fig. 1; gravity \times parity, $F [1,56] = 4.815$, $P < 0.05$; NK test, $P < 0.05$). Two times more primigravid HG dams lost neonates from their litters

TABLE 1. Number of pregnancies, neonates born, average litter size, birth mass and gender ratio of offspring born to rat dams following exposure to various gravity treatments.

Treatment group	No. pregnant dams	No. neonates*	Litter size* ($\bar{x} \pm$ SD)	Birth weight [†] (g) ($\bar{x} \pm$ SD)	Gender ratio [‡] (M/M+F)
HG	30/32	357	12.3 \pm 2.8	6.0 \pm 0.6 ^a	0.52
SYC	30/32	364	12.6 \pm 2.5	6.9 \pm 0.4 ^b	0.49
RC	8/8	76 [§]	13.0 \pm 1.3	6.3 \pm 0.4 ^a	0.51
VIV	16/16	208	13.0 \pm 2.3	6.9 \pm 0.4 ^b	0.51

* All neonates (live and dead); data calculated from litter totals.

[†] Live neonates only; data derived from litter averages. Means with different superscripts are different ($P < 0.05$).

[‡] Live neonates only; data derived from litter totals.

[§] Total progeny from seven dams only. One dam was eliminated because of a rapidly growing abdominal mass.

than did bigravid (B) HG dams (HG dams showing neonatal mortality: P, 66%; B, 33%; $\chi^2 [1] = 3.33$, $P < 0.07$). The number of P0 losses was not related to litter size ($R^2 = 0.14$, NS).

Postnatal Survival

Log survivorship functions (Fig. 2) derived from the HG groups show that whereas primigravid dams had fewer survivors within their litters as compared with bigravid dams, there were no differences in neonatal survival on subsequent days ($F [6,84] = 4.36$, $P < 0.001$; P1, NK test, $P < 0.05$; all other comparisons, NS). Neonatal losses on P0 were not predictive of subsequent postnatal losses on any day from P1 to P7 (R^2 s < 0.10 , NS) although neonatal birth weight was significantly related to pup survival during this interval ($R^2 = 0.18$, $P < 0.05$).

Litter sizes stabilized on P7. A subset ($n = 8$) of HG litters centrifuged from G11 until P21 for purposes of a second experiment (unpublished results) showed no mortality during the period spanning P7 to P21.

Postnatal Body Mass

Figure 3 illustrates postnatal body masses for a subset of HG and SYC pups on P0, P2, and P5. ANOVA revealed

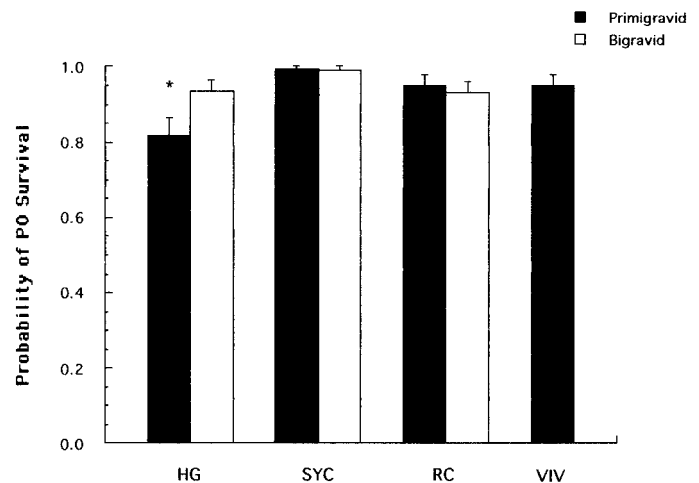
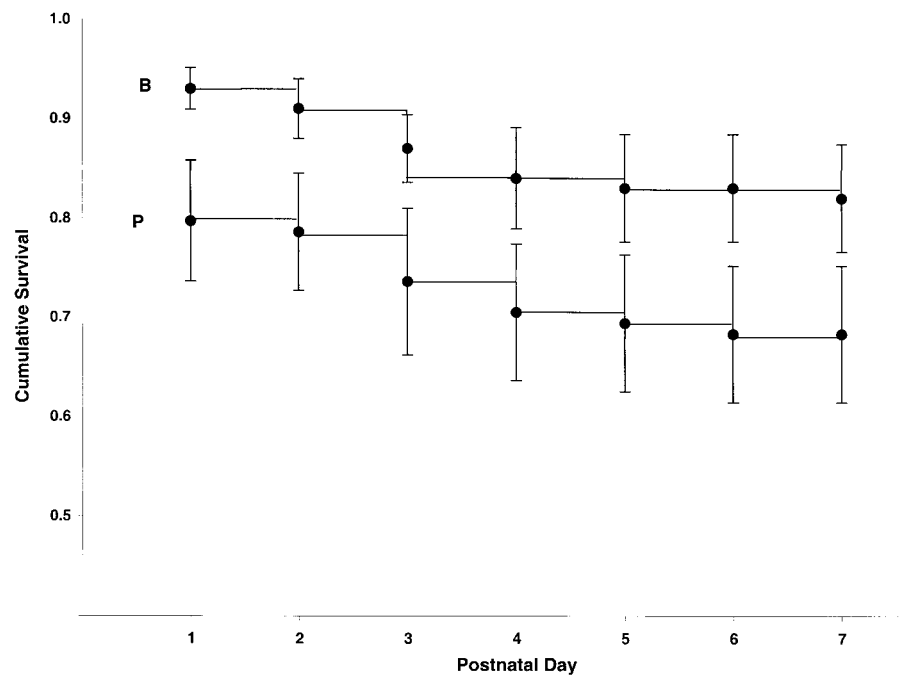


FIG. 1. Percentage of live neonates found in the HG, SYC, RC, and VIV litters on the day of birth (P0) as a function of maternal parity ($*P < 0.05$). For the HG and SYC treatments, sample sizes are 15 rats/parity condition. For the RC treatment, litters were derived from four primigravid and three bigravid dams. For the VIV treatment, $N = 16$ (primigravid condition only).

FIG. 2. Log survivorship function depicting cumulative postnatal mortality (\pm SEM) from Postnatal Day 1 (P1) to P7 in hyper-gravity-reared litters ($n = 15$) as a function of maternal parity. (P, primigravid; B, bi-gravid). There were no statistical differences between parity conditions following the day of birth (P0).



that HG pups weighed significantly less than SYC pups at all three postnatal ages ($F [1,30] = 62.82, P < 0.0001$), with no significant effect of parity.

Maternal Body Weight

Comparisons of maternal body weight in the HG, RC, and SYC conditions prior to birth (on G22) and during the first postnatal week (P0, P3, and P5) for the dams that were studied postnatally are shown in Figure 4. A main effect of day ($F [3,108] = 351.3, P < 0.0001$) and a condition \times day interaction ($F [6,108] = 2.8, P < 0.01$) emerged from this analysis. We observed a nonsignificant trend toward an effect of gravity ($F [2,36] = 3.0, P < 0.06$). Post hoc analyses indicated that on G22, P0, and P2, HG dams weighed significantly less than the SYC dams, whereas the RC

group did not differ from either the HG or SYC groups. On P5, there were no group differences.

In light of the G22 group differences, we determined whether proportions of the dam's body mass accounted for by litter mass differed across conditions. We adjusted neonatal birth mass for dam body mass (per 100 g body weight) by estimating average litter birth masses. Nonsurviving neonates were assigned the average body mass of their siblings. (Although neonates found dead following parturition were not individually weighed, their gross body sizes did not differ noticeably from those of survivors.) This analysis revealed that across gravity conditions litter mass accounted for similar proportions (mean \pm SD) of dam body mass (HG: P, 22.8% \pm 5.4%, B, 21.2% \pm 2.3%; SYC: P, 24.7% \pm 6.2%, B, 23.0% \pm 1.6%; RC: P, 24.4% \pm 2.1%, B, 22.1% \pm 4.5%).

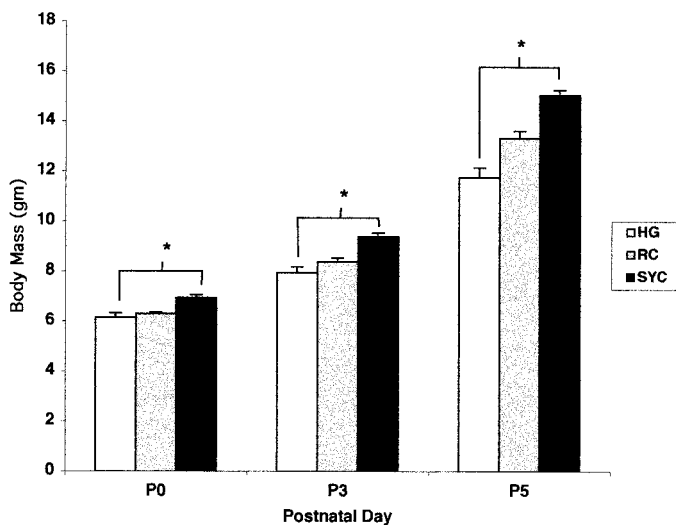


FIG. 3. Body mass (in grams; mean \pm SEM) of HG, SYC, and RC pups at P0, P2, and P5. For HG and SYC, sample sizes are 30 litters/condition; for RC, sample sizes are 7 litters/condition. * $P < 0.05$. Data are collapsed across parity conditions.

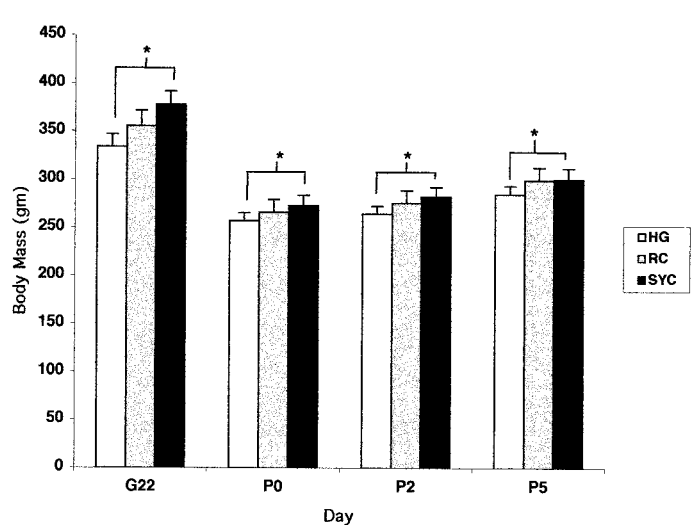


FIG. 4. Body mass (in grams; mean \pm SEM) of HG, SYC, and RC dams at G22, P0, P2, and P5. For HG and SYC, sample sizes are 30 litters/condition; for RC, sample sizes are 7 litters/condition. * $P < 0.05$. Data are collapsed across parity conditions.

TABLE 2. Maternal behavior during parturition and the first three postpartum hours in hypergravity-exposed ($1.5 \times g$) primigravid (P) and bigravid (B) dams relative to $1.0 \times g$ controls.*

Behavior	Condition				Significance [†]		
	HG		SYC		Gravity	Parity	Interaction
	P	B	P	B			
Parturition (min/duration of parturition)							
Duration	106.0 ± 57.2	108.3 ± 44.0	98.3 ± 42.6	106.8 ± 41.2	NS	NS	NS
Lick/handle	35.1 ± 15.6	38.0 ± 26.6	34.6 ± 8.1	37.5 ± 10.9	NS	NS	NS
Pup contact	104.8 ± 56.1	107.7 ± 42.8	98.1 ± 42.2	106.6 ± 41.2	NS	NS	NS
Placentophagia	22.1 ± 13.1	14.8 ± 6.2	14.4 ± 6.8	20.0 ± 9.3	NS	NS	NS
Postparturition (min/180 min)							
Self-grooming	16.4 ± 10.4	18.4 ± 12.2	21.6 ± 4.0	22.0 ± 5.5	NS	NS	NS
Lick/handle	28.9 ± 10.4	30.2 ± 10.1	24.1 ± 16.9	26.9 ± 12.8	NS	NS	NS
Nursing	139.8 ± 8.4	143.4 ± 15.2	128.6 ± 17.0	132.4 ± 12.9	NS	NS	NS
Pups out of nest	37.8 ± 9.9	23.9 ± 7.9	10.0 ± 6.0	18.5 ± 4.3	<0.05	NS	<0.05
Digging in nest	26.5 ± 10.9	11.5 ± 2.1	5.8 ± 1.0	9.1 ± 2.0	<0.05	NS	<0.05

* n = 4 dams/condition.

† Newman-Keuls post hoc test.

Maternal Behavior During Parturition and the Initial Postpartum Period

Video analyses revealed that the postures and general behaviors of the HG and SYC dams were virtually indistinguishable. During parturition, HG dams in both parity conditions displayed characteristic maternal care, licking and cleansing each pup as it emerged from the birth canal, and retrieving neonates from outside the nest. They licked and handled newborns for similar amounts of time as compared with controls (Table 2; gravity, $F [1,12] = 0.00$, NS; parity, $F [1,12] = 0.09$, NS) and spent comparable periods in direct contact with their pups (NS). Placentophagia also did not differ across groups (gravity, $F [1,12] = 0.08$, NS; parity, $F [1,12] = 0.04$, NS). The duration of parturition, as measured from the time of birth of the first pup to the time of nursing onset, did not differ across conditions. All of the dams began to nurse pups soon after the birth of the last pup. During the 3 h immediately following parturition, observations were made of maternal self-grooming, licking and handling pups, litter distribution within and outside of

the nest, and digging (Table 2). The results of this analysis revealed that primigravid HG mothers tended to disrupt nursing bouts and the huddle by frequently digging within and rearranging the nest (gravity, $F [1,11] = 14.1$, $P < 0.01$; gravity \times parity, $F [1,11] = 9.0$, $P < 0.01$). In contrast to the other conditions, among HG litters the presence of one or two pups outside of the nest was more frequently observed (gravity, $F [1,11] = 15.1$, $P < 0.01$; gravity \times parity, $F [1,11] = 6.3$, $P < 0.01$). A simple regression was performed to ascertain the relationship between the mothers' behaviors and postpartum mortality. Maternal agitation during nursing was negatively correlated with neonatal survival ($R^2 = 0.99$, $P < 0.001$), and the amount of time that one or two pups were observed outside of the nest was negatively correlated with neonatal survival ($R^2 = 0.99$, $P < 0.02$).

Maternal Behavior During the First Postnatal Week

Analyses of the dams' maternal care patterns on P0, P3, and P5 are shown in Table 3. Dams in both gravity and

TABLE 3. Maternal behavior of lactating hypergravity-exposed ($1.5 \times g$) primigravid (P) and bigravid (B) dams relative to $1.0 \times g$ controls.*

Behavior	Condition				Significance [†]		
	HG		SYC		Gravity	Parity	Interaction
	P	B	P	B			
Licking (min/720 min)							
P0	20.6 ± 10.1	15.0 ± 5.5	18.9 ± 5.2	19.9 ± 3.5	NS	NS	NS
P2	16.4 ± 5.2	11.9 ± 2.3	18.9 ± 3.7	15.6 ± 6.4	NS	NS	NS
P5	16.1 ± 10.8	17.7 ± 3.5	18.6 ± 3.9	18.0 ± 1.2	NS	NS	NS
Retrieving (min/720 min)							
P0	3.7 ± 10.1	2.3 ± 1.7	1.2 ± 5.2	2.2 ± 1.2	NS	NS	NS
P2	3.5 ± 3.6	2.7 ± 1.5	0.3 ± 3.7	1.1 ± 0.7	NS	NS	NS
P5	3.7 ± 3.7	3.2 ± 3.1	0.2 ± 3.9	0.4 ± 0.4	NS	NS	NS
Nursing (h/12 h)							
P0	8.4 ± 0.3	9.9 ± 0.4	7.5 ± 0.3	6.7 ± 0.1	<0.05	NS	<0.05
P2	8.3 ± 4.7	8.5 ± 0.4	7.1 ± 0.9	6.2 ± 0.5	<0.05	NS	NS
P5	8.0 ± 0.8	7.3 ± 0.7	5.0 ± 1.0	5.2 ± 0.6	<0.05	NS	NS
Non-pup-directed maternal behavior (min/720 min)							
P0	70.3 ± 20.5	19.7 ± 4.2	0.5 ± 0.4	22.3 ± 10.7	<0.05	NS	<0.05
P2	63.8 ± 24.7	23.3 ± 11.8	2.9 ± 2.2	11.8 ± 9.9	<0.05	NS	NS
P5	32.2 ± 19.2	16.8 ± 14.7	0.0 ± 0.0	0.1 ± 0.1	<0.05	NS	NS

* n = 4 dams/condition.

† Newman-Keuls post hoc test.

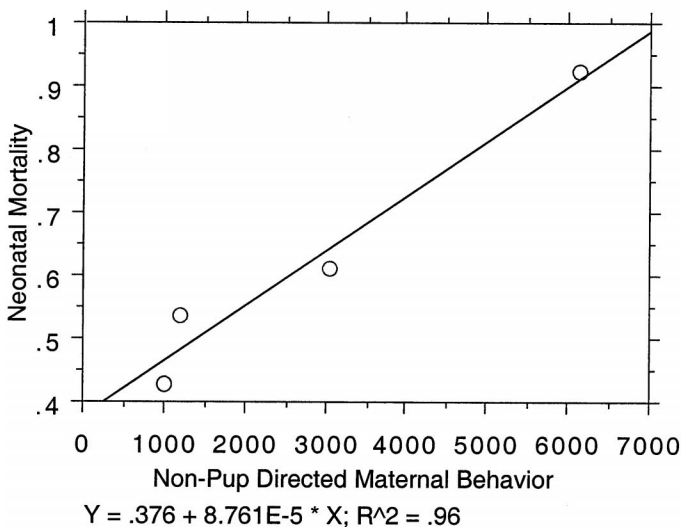
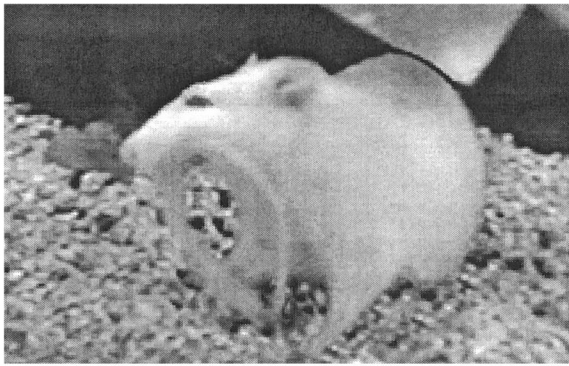


FIG. 5. Top: Non-pup-directed maternal behavior in a primigravid rat dam on the first postnatal day. Note the neonates in nest on the right side of the image. Bottom: Regression plot showing the relationship between non-pup-directed maternal behavior and neonatal mortality in postparturient primigravid rat dams.

parity conditions displayed equivalent amounts of pup licking (HG P0: 21.6 ± 10.1 min; gravity: $F [1,12] = 2.914$, NS; parity, $F [1,12] = 0.959$, NS) and retrieving (gravity: $F [1,12] = 2.839$, NS; parity, $F [1,12] = 0.202$, NS). Compared with SYC dams, HG dams spent significantly greater proportions of their time nursing ($F [1,12] = 23.21$; $P < 0.001$). An interaction of gravity \times parity \times day revealed that on the day of birth, primigravid HG dams spent significantly less time nursing than did bigravid HG dams but spent significantly more time nursing than did SYC dams ($F [3,36] = 2.92$, $P < 0.05$, NK test, $P < 0.05$). Non-pup-directed maternal behavior, characterized by incessant tail retrieving and nest building (Fig. 5), was observed at high levels in the HG dams with the greatest amounts observed in primigravid HG dams on the day of birth (Table 2; gravity \times parity, $F [1,12] = 8.9$, $P < 0.01$). Simple regression revealed a significant linear correlation between non-pup-directed maternal behavior and neonatal deaths during the first 24 h postpartum (Fig. 5; $R^2 = 0.96$, $F [1,3] = 47.6$, $P < 0.02$). There was no relationship between the protracted nursing bouts and neonatal survival ($R^2 = 0.14$, NS). On P2 and P5, levels of non-pup-directed maternal behavior

and nursing were elevated in the HG dams relative to the SYC dams for both parity conditions (P2, $F [6,136] = 0.000$, $P < 0.03$; P5, $F [1,12] = 4.085$, $P < 0.06$, NS).

DISCUSSION

Together, the results reported herein specify the effects of mild hypergravity ($1.5 \times g$) exposure during pregnancy, birth, and lactation on neonatal outcome. Our analyses spanned the rats' ontogenetic trajectory throughout the postnatal nursing period, i.e., from the day of birth throughout the first postpartum week. Overall neonatal losses at $1.5 \times g$ were modest, with the vast majority of offspring surviving the suckling period. Maternal parity was a determinant of early postpartum survival of neonates that underwent late gestation and birth at $1.5 \times g$. However, following the day of birth, neonatal mortality in hypergravity was independent of maternal reproductive experience. During hypergravity exposure, neonatal well-being at each of these distinctive ontogenetic periods (early postpartum vs. postnatal) may be determined by different, possibly unrelated mechanisms.

The increased gravitational load to which dams were continuously exposed beginning on G11 did not interrupt pregnancy or birth. Pregnancies were carried to term in equivalent numbers across experimental conditions, and hypergravity-exposed dams gave birth within the expected temporal interval to robust, well-formed neonates. Litter sizes were identical in numbers relative to those of SYC dams, although neonatal mortality at birth was selectively elevated in the primigravid HG litters. The absence of fetal wastage among HG dams is consistent with earlier reports that mid- and late gestation mammalian embryos readily survive and develop during exposure to variations of about $1 \times g$ relative to the earth's gravity [24, 25, 36]. Our findings are also consonant with studies in which young rodents have conceived, given birth, and reared their postnatal offspring in hypergravity [26, 36, 37]. We observed neonatal survival rates similar to those reported by Oyama et al. [36] at $1.27 \times g$ and $1.48 \times g$ (85% and 96%, respectively) for dams impregnated while undergoing centrifugation. This similarity is noteworthy because the rat dams in the present experiment were adapted to centrifugation for a much briefer period (from G11).

The most striking finding of the present experiment is the sharp increase in neonatal mortality among hypergravity-exposed primigravid mothers as compared with bigravid mothers. Video observations of parturition revealed that the hypergravity-specific offspring deaths occurred postnatally rather than prenatally and did not appear to be related to the mother's treatment of the pups during parturition. No differences were observed in the amounts of time that dams in all four treatments spent licking and retrieving their pups, in contact with pups, or engaged in placentophagia. The duration of parturition, measured from the birth of the first pup until the mother crouched over the pups to provide access to her nipples, was comparable across groups (Table 2). Together, these observations rule out disturbances in the mothers' parturition behavior similar to those that have been reported following infraorbital denervation [38] or olfactory bulbectomy [39]. In these reports, alterations in sensory processing led to notable impairments in delivery, removal of the amniotic sac, nest building, retrieving, and grouping of pups and delayed nursing onset.

Observations made during the first three postpartum hours revealed that HG dams, particularly primigravid dams, repeatedly interrupted nursing bouts by disrupting

the bedding and the huddle. As compared with bigravid HG dams and controls, inexperienced dams tended to be less effective in keeping pups grouped within the nest. Both of these factors were highly correlated with neonatal mortality. Denenberg et al. [40, 41] presented data showing that what transpires between the mother and the pups during the first 12 h of life has a significant impact on neonatal survival and growth. The initial postpartum period is characterized by rapid growth and transformation of major organ systems. Neonatal rats that spent the first postnatal hour with a dam that had been lactating for 10 days rather than a newly parturient dam showed reduced survival and weight gain. The mother's behavior toward her pups appears to be the critical variable in determining neonatal survival and weight gain, the latter changes persisting throughout weaning. Whereas newly parturient females quickly settled over the newborn pups, becoming quiescent, and allowing the young to rapidly initiate nursing, the 10-day lactator was very active, moving about the cage, licking the pups frequently, and self-grooming. These observations are relevant to the present findings. We identified repeated disturbances of nursing episodes, particularly by HG primigravid dams, that may have produced effects similar to placing newborns with a dam of discordant developmental status.

The log survivorship functions (Fig. 2) show that neonatal losses from P1 to P7 were comparable across parity conditions. Thus, maternal experience provided no immunity against neonatal mortality after the first 24 h postpartum. One interpretation of this finding is that endocrine and experiential changes during parturition, coupled with initial postpartum exposure to neonates, was sufficient to equilibrate the maternal behavior of primigravid dams with that of bigravid dams. However, the absence of a parity effect after P0 does not rule out maternal contributions to postnatal losses. Maternal factors may play a role in postnatal mortality after P0 but not via a parity-dependent mechanism. The postnatal video recordings revealed that HG dams of both parity conditions spent significantly greater amounts of time than SYC dams engaged in non-pup-directed maternal behaviors and in nursing. The increased amounts of time that HG dams spent crouched over pups may reflect the dam-initiated activities that disrupted nursing bouts, leading to compromised milk intake by the neonates. Even brief interruptions of the nursing relationship are likely to alter the process of milk transfer to pups, possibly leading to reductions in the amounts transferred. Thus, nutritional deficits during the first postnatal week may account for the neonatal losses. The strong linear correlation between the non-pup-directed maternal behaviors and postpartum mortality (Fig. 5) also raises the possibility that the dams' activities involved vigorous, incessant activity leading to trauma and crush injuries if the pups are trampled. Hematomas and other external signs of physical injury were not detected in any of the neonates.

Very little is known about the reproductive endocrinology of females exposed to altered gravity. Burden et al. [42] reported no change in estrogen or progesterone levels in pregnant females flown in the microgravity environment of space. Megory and Oyama [26] reported reduced prolactin levels in periparturient dams exposed to gravitational loads spanning $1.76 \times g$ to $4.17 \times g$ relative to $1.0 \times g$ controls. Prolactin plays a major role in the induction and maintenance of maternal behavior [21]. At higher gravitational loads, alterations in maternal care that could account for neonatal losses during centrifugation might be more clearly observed. Clearly, additional data are needed to un-

derstand gravity-specific effects on female reproductive hormones and consequences for maternal caregiving responses.

Birth weights of the HG and RC offspring were reduced relative to the SYC and VIV offspring, revealing an effect of centrifugation on prenatal growth that was not specific to hypergravity but rather was common to the centrifuged groups. A number of possibilities can account for the reduced birth weights, including restricted placental blood flow and alterations in fetal growth hormone [43]. Prenatal maternal stress is also associated with low birth weight [44], although it is unlikely that the modest gravitational load utilized in this study produced significant stress in the dams. Research on adult male rats has shown that even at $2.0 \times g$ corticosterone levels are initially elevated at the onset of centrifugation, but the effect subsides after the fourth day following the onset of centrifugation [45]. The dams in the present study were well adapted to centrifugation by G15, a full week prior to parturition. We recently reported initial reductions in body mass, food and water intake, and activity levels in dams exposed to centrifugation throughout pregnancy and weaning, i.e., from G11 to P21 [22]. However, food and water intake (adjusted per 100 g of body mass) did not differ in hypergravity-exposed and control dams after the fourth day of centrifugation. In the present study, analyses of P0 litter mass as a proportion of maternal body mass prior to birth eliminated neonatal body weight differences across groups. This finding indicates that following gestation in hypergravity neonatal birth weights are precisely scaled to dam body weights. Birth weights were not significantly related to P0 deaths; however, birth weights were inversely related to postnatal losses from P1 to P7. This observation further supports the view that differing mechanisms account for neonatal mortality immediately following birth versus later during the postnatal period. Because we did not cross-foster neonates to noncentrifuged dams, it was not possible to differentiate among prenatal and postnatal maternal contributions to these findings. It was also beyond the scope of the present study to analyze the relative attractiveness to the dams of hypergravity-reared versus noncentrifuged neonates. Systematic manipulation of each of these factors is likely to provide data useful for identifying sources of neonatal death in hypergravity. Clearly, additional studies of the effects of centrifugation on the maternal-fetal system are needed to test specific hypotheses of the role of gravity in prenatal growth and well-being and to further differentiate among maternally and nonmaternally mediated effects of gravity on the fetus and neonate.

Maternal body weight, a function of maternal age, differed for primigravid and bigravid dams. Thus, advanced body size and strength associated with the dam's maturity, rather than parity, may have accounted for the observed differences in neonatal mortality in the litters of experienced and inexperienced dams. However, there is little evidence to support this hypothesis. First, maternal body masses were not related to neonatal losses on P0. Second, the effect of maternal experience was transient, apparent only on the first postpartum day. Age and size differences in maternal care would be expected to persist over days. Together, these considerations suggest that parity, rather than the size and strength of the experienced dams, accounted for differences in mortality at birth in neonates born to primigravid and bigravid dams.

The differential survival probabilities of neonates born to primigravid versus bigravid dams in the hypergravity

environment reveal are startling. Whereas other researchers have reported characteristic neural, endocrine, and behavioral differences among mothers of differing parities [1, 4, 5], this is the first time an influence of maternal reproductive experience on offspring survival has been identified in rodents. Changes in maternal behavior patterns were strongly correlated with neonatal mortality. Adaptation of adult rodents to hypergravity occurs within approximately 4 days, after which time initial reductions in food and water intake and body mass subside and transient elevations in corticosterone levels return to normal [22, 45]. In our study, the period of adaptation to centrifugation occurred from G11 to G15, not around the time of birth (G22/23). However, the combined stresses of hypergravity exposure and birth may have triggered the expression of these parity-related differences in maternal care and neonatal survival. The results of our study suggest that seemingly minor disruptions in maternal behavior patterns can result in major influences on neonatal outcome. These findings support the general hypothesis that a close synchrony must exist between the mother and infant at birth to produce adequate postnatal survival and development. Under atypical environmental circumstances such as those imposed by increased gravity, changes in maternal care patterns erode the synergistic fit that characterizes maternal-offspring relations. Reproductive experience protects against effects of environmental change that may otherwise disrupt maternal care patterns and lead to neonatal compromise and even death.

The present findings suggest that during the perinatal period (the period around the time of birth), the maternal-offspring system is especially sensitive to relatively modest ($0.5 \times g$) increments in the Earth's gravitational field. These findings, derived from reproductively experienced and inexperienced rat mothers and their offspring exposed to $1.5 \times g$ hypergravity, implicate maternal caregiving in initial neonatal survival at birth. The neonate is profoundly vulnerable to changes in the Earth's gravitational field, potentially through direct as well as indirect influences (i.e., those arising through maternal avenues). In the present study, we identified maternal reproductive experience as a major determinant of postpartum survival under altered gravitational conditions. The role of maternal factors cannot be underestimated in studies through which we seek to ascertain gravity's role in mammalian reproductive and ontogenetic processes. Maternal-offspring interactions under altered gravitational conditions warrant further investigation to further our understanding of the influence of gravity on developing mammals. As humans move closer to living and working in space, a clearer understanding of how early life events and developmental trajectories unfold in hypergravity is important for establishing a basis for reproduction and development on space platforms.

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