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Effects of acute hypergravity exposure and parity on maternal behavior in CD-1 mice

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Abstract. We assessed the behavioral response to acute hypergravity exposure in lactating mother mice, *Mus musculus* of the outbred CD-1 strain. Primiparous or terziparous dams were exposed with their litters to a centrifugal force equivalent to 2G hypergravity for 1 h daily from postnatal day 2 (P2) to P9. We made detailed behavioral observations before, during and after the rotation on selected days to identify elements of the maternal behavioral repertoire vulnerable to 2G challenge. Licking and nest building were reduced during rotation while mothers sniffed and snouted their pups more. Nursing and total time in physical contact with pups were relatively stable. The effects of rotation were most pronounced on P2, dams appearing to habituate to the treatment with repeated exposure. Dam parity had a limited effect on the behavioral response to rotation, primiparous mothers tending to spend longer nursing their pups during the rotation and showing a greater tendency to lick and nest-build in post-rotation. Differences between parity groups diminished over days. Body weight was decreased in rotated primiparous dams and their pups gained less weight than stationary controls. Ultrasonic vocalization (USV) rates recorded on P2, P5 and P9 seemed to indicate delayed behavioral development in rotated pups.

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INTRODUCTION

The conquest of space, numbering among its pioneers the she-dog Laika whose 1957 mission was followed four years later by Yuri Gagarin, has witnessed a growing variety of both vertebrate (tadpoles, frogs, rats, mice, etc.) and invertebrate (flies, scorpions, protozoa, etc.) species in zero gravity (0G). With the approaching readiness of the International Space Station (a 16-nation joint enterprise) the number of humans undertaking space travel is predicted to rise, making studies which improve our understanding of the biological impact of gravity increasingly important.

An altered gravitational environment represents a unique challenge for biological systems that have evolved against a constant gravitational background. Ground-based experiments use G-loads generated by centrifugation with the expectation that behavioral and physiological reactions to hypergravity help to explain reactions to the microgravity challenge faced by orbiting animals. Thus hypergravity models can be used to proactively anticipate potential health problems for both humans and animals in space. Disruptions in biological processes that occur upon exposure to an altered gravitational environment may also shed light on their normal regulation and integration.

Progress in gravitational research on animals will be contingent on a mother-offspring unit that is reliably functional in altered gravity (Alberts and Ronca 1999). Continuous exposure to hypergravity is known to diminish early postnatal survival in rat pups (Ishay and Barr-Nea 1977, Megory and Oyama 1984, Oyama and Platt 1967) and in 1998 around 50% of eight-day-old rats failed to survive a two-week long space mission (Reichardt 1998). A series of studies have been aimed at investigating the physiology and behavior of pregnancy delivery and early postnatal mother-pup dyad (Plaut et al. 1999, Ronca and Alberts 2000, Ronca et al. 2000) a focus which may also shed light on the regulation of maternal behavior in rodents by disentangling elements in the maternal pattern.

Mice and rats are altricial species whose young are entirely dependent on effective maternal care during the first few weeks of life (for extensive review see Rosenblatt and Lehrman 1963). Previous maternal experience is believed to contribute to qualitative and quantitative changes in the maternal repertoire (for example see Noirot 1972), experienced females being more responsive to pup stimuli (Bridges et al. 1972, Cohen and Bridges 1981, Cohen-Salmon et al. 1982, Noirot 1974, Orpen and Fleming 1987) and Ronca and coauthors (1999) reported that parity counteracted hypergravity-induced pup mortality in the first week postnatally for rats exposed to continuous centrifugation.

Laboratory mice, by merit of their small body size, are eminently suitable animal models for research in space. Moreover, neonatal mice reportedly tolerate chronic hypergravity better than rats (Oyama and Platt 1967).

The aim of the present study was to characterize the behavioral response of mother mice upon acute exposure to rotationally-generated hypergravity. Few data are in fact available on short term or repeated exposure to changes in gravitational environment. These kind of exposure, which is generally aimed to gain insight on the effect of short episodes of hypergravity mimicking some effects of launch and re-entry into and from space (Le Bourg 1999), may also evaluate specific vulnerability and identify elements of the maternal repertoire vulnerable to this environmental stress.

To this purpose, we made fine grain behavioral observations before, during and after an hour of centrifugation. Moreover, to determine the relevance of parity in the mother's behavioral response to rotationally-generated hypergravity, we compared primiparous and terziparous mothers. Finally, we monitored pup body-weight and ultrasonic vocalization rate (USV) in rotating (experimental group) and non-rotating (control group) pups to evaluate the possibility of pup-mediated effects of rotation on the mother's behavior.

METHODS

Animals

We used mice of the outbred Swiss-derived CD-1 strain obtained as young adults from Charles River Italia (Italy). On arrival animals were housed in standard wire-topped Plexiglas cages (42 × 27 × 14 cm) with up to 10 individuals per cage. Sawdust (pine shavings type 20 purchased from Allevamenti Plaisant, Italy) was used as bedding. The colony room was maintained at 21°C and 60% relative humidity on a 12/12-hour reversed white-light/red-light cycle (red lights on at 08:30 A.M.). Water and pellet food (Enriched Standard Diet purchased from Mucedola, Italy) were available *ad libitum*.

Apparatus

The centrifuge was custom-made by Isolceram (Italy). It consisted of a circular aluminum table mounted on a central motor. The table was 1 m in diameter and fitted to accommodate up to six home cages (33 × 13 × 14 cm) around its circumference. During the rotation phase of the experiment we rotated the turntable at a constant rate of 56 rpm (angular velocity 336°/s) to create a linear acceleration of 2G inside the home cages. In order to have the cage floor perpendicular to the gravity, a wood wedge was putted under each cage. We used video cameras (Sony Digital Handycam DCR-TR7000E) fixed to the table to record the mice's behavior.

Breeding procedure

We moved young adult females in pairs to smaller cages (33 × 13 × 14 cm) where they remained together with a male for 15 days. Pregnant animals we thereafter housed individually and provided with 1.5 g of shredded tissue as nesting material. They usually delivered around six days later. We checked dams for delivery daily at 11:00 A.M. and culled litters to six (three females, three males) 24 hours after noting parturition. The terziparous group underwent two cycles of pregnancy and lactation in preparation for the experiment. Their pups were weaned on postnatal day (P) 21 (day of birth = P0) and they were returned to group cages and rested for at least 20 days.

The study involving experimental animals has been carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Experimental procedure

Our subjects were 10 primiparous and 10 terziparous females and their litters. The experimental procedure was repeated daily for a total of eight days (P2 to P9 inclusive). We took mother and litter in their home cage from the colony room at 11:00 A.M. and secured them to the centrifuge where they remained for three hours; one hour stationary (Pre-rotation), one hour rotating (Rotation) and a further hour stationary (Post-rotation). We then removed the home cage from the table, weighed dam and recorded pup body weights and USVs (see below) before returning animals to the colony room. Video recordings of the mice over the three experimental hours were taken on P2, P4, P6 and P9, days representing key stages in pup brain/behavioral development (Alleva et al. 1989, Fox 1965).

Behavioral observations

We scored the dams' behavior using a keyboard event recording system feeding to a computer for analysis (The Observer 2.0, Noldus). A 7-minute focal sample was taken from the beginning, middle and end of every hour of the experimental procedure. Throughout these samples we recorded the mother's position in the home cage relative to the pups and her behavior according to the categories listed below (Fleming and Sarker 1990, Laviola et al. 1994, Noirot 1965). Mother-pup contact categories are mutually exclusive and exhaustive, as are behavioral items.

Mother-pup contact categories

Full Ventral Contact: dam is positioned so that her ventral surface is in full contact with all her pups, she is usually crouched above them or lying slightly on her side next to them thus allowing maximum access to her nipples, her limbs are often splayed to further accommodate the pups. Partial Contact: dam's body is in physical contact with the pups but one or more of the pups do not have full access to her ventral surface. No Contact: dam's body is not in physical contact with the pups (though she may be sniffing, snouting, licking or retrieving them).

Behavioral items

PUP DIRECTED BEHAVIORS

Sniff: sniffing the pups; Snout: nudging the pups with snout; Retrieve: carrying a pup in mouth, usually engaged in transferring it from one part of the cage to another; Lick: licking the pups, usually concentrating on their ano-genital region; Nest-build: moving the nesting material or sawdust to construct or rearrange the nest, sometimes plunging head and shoulders into the sawdust among the pups; Nurse: positioned over the pups in the lactation position and otherwise inactive, inattentive to surroundings, head often resting with eyes partly or fully closed.

OTHER BEHAVIORS

Active: moving about the cage floor and/or making exploratory head movements and sniffing at the sawdust, nest material, cage or air; Rear: standing on hind legs, front paws may rest on walls; Bar-hang: moving about on the barred ceiling of the cage, no paws resting on the floor; Still: remaining completely still with eyes open; Self groom; Eat; Drink; Sleep; self explanatory; Sawdust-root: rooting in the sawdust with snout or front paws, often eating pieces of sawdust or feces; Dig: digging frantically in the sawdust, kicking with fore or hind legs so that it sprays or burrowing in it with head and shoulders with no obvious nest arranging or sawdust-rooting function; Tail-chase: taking own tail in mouth and starting to carry it towards the nest, often repeating this action several times; Nest (away): attempting to lodge pieces of nesting material, sawdust or feces between the bars of the ceiling.

Body weights and pup USVs

We monitored a non-rotating control group of 10 primiparous dams and their litters alongside the primiparous rotated group to test for effects of rotation on pup development. Whilst the experimental group was secured to the centrifuge the control group was instead placed on an adjacent surface where they were similarly exposed to the environs and noise of the centrifuge. Treatment of the two groups was otherwise identical.

We made measurements of pup USVs at the end of the 3-hour procedure on P2, P5 and P9 (Brennan et al. 1999). A pup of each sex was randomly selected from the nests of six primiparous rotated litters and six primiparous control litters and placed in a Petri dish lined with tissue. After waiting 15 seconds we counted USVs emitted during two consecutive minutes using a hand held bat detector (Ultrasound Advice, model S25). In addition, we weighed all mothers and all their pups to the nearest 0.1 grams (Mettler PK-300 balance correcting for body movements) after every 3-hour procedure.

Statistical analyses

We analyzed behavioral data using a mixed model ANOVA considering parity as a grouping factor and phases and days as repeated measures. To analyze dam weight we used an ANOVA considering treatment as a grouping factor and days as repeated measures. To analyze pup weights and USVs we used a mixed model ANOVA with treatment as grouping factor, litter as random blocking factor and sex and days as repeated measures. We made post hoc analyses using the Tukey test.

RESULTS

Mother-pup contact

As days progressed, duration of Full Ventral Contact increased in all dams ($F_{3,54}$=5.64, P<0.05) (see Fig. 1A). Partial Contact was concomitantly reduced though this trend was not statistically significant ($F_{3,54}$=2.51, P=0.0686). Within days contact levels changed over rotational phases; duration of Full Ventral Contact decreasing upon rotation and remaining suppressed afterwards ($F_{2,36}$=7.87, P<0.05) while Partial Contact concurrently increased then tended to fall back towards Pre-rotational levels ($F_{2,36}$=7.12, P<0.05).

Furthermore, results suggest that parity is relevant to how these phase effects change over days. On P2 all mothers maintained a steady level of Full Ventral Contact throughout the procedure. However, on successive days terziparous dams always reduced Full Ventral Contact during Rotation while primiparous did not do likewise until P9. Similarly, terziparous mothers increased Partial Contact during Rotation on every day of observation while primiparous did so only from P6 onwards.

Duration of No Contact showed few significant changes throughout the experiment. However, while primiparous dams showed no fluctuations in levels of No Contact throughout the experiment, terziparous had a more irregular profile, reducing No Contact during Rotation on P2 and increasing No Contact during Rotation on P6 (parity × day × phase: $F_{6,108}$=3.22, P<0.05).

Behavioral items

CHANGES OVER DAYS

Performance of all pup-directed behaviors other than licking, as well as several non-maternal behaviors, changed over days ($F_{3,54}$ for duration and frequency respectively: Sniff = 4.23, 7.46; Snout = 3.36, 4.95;

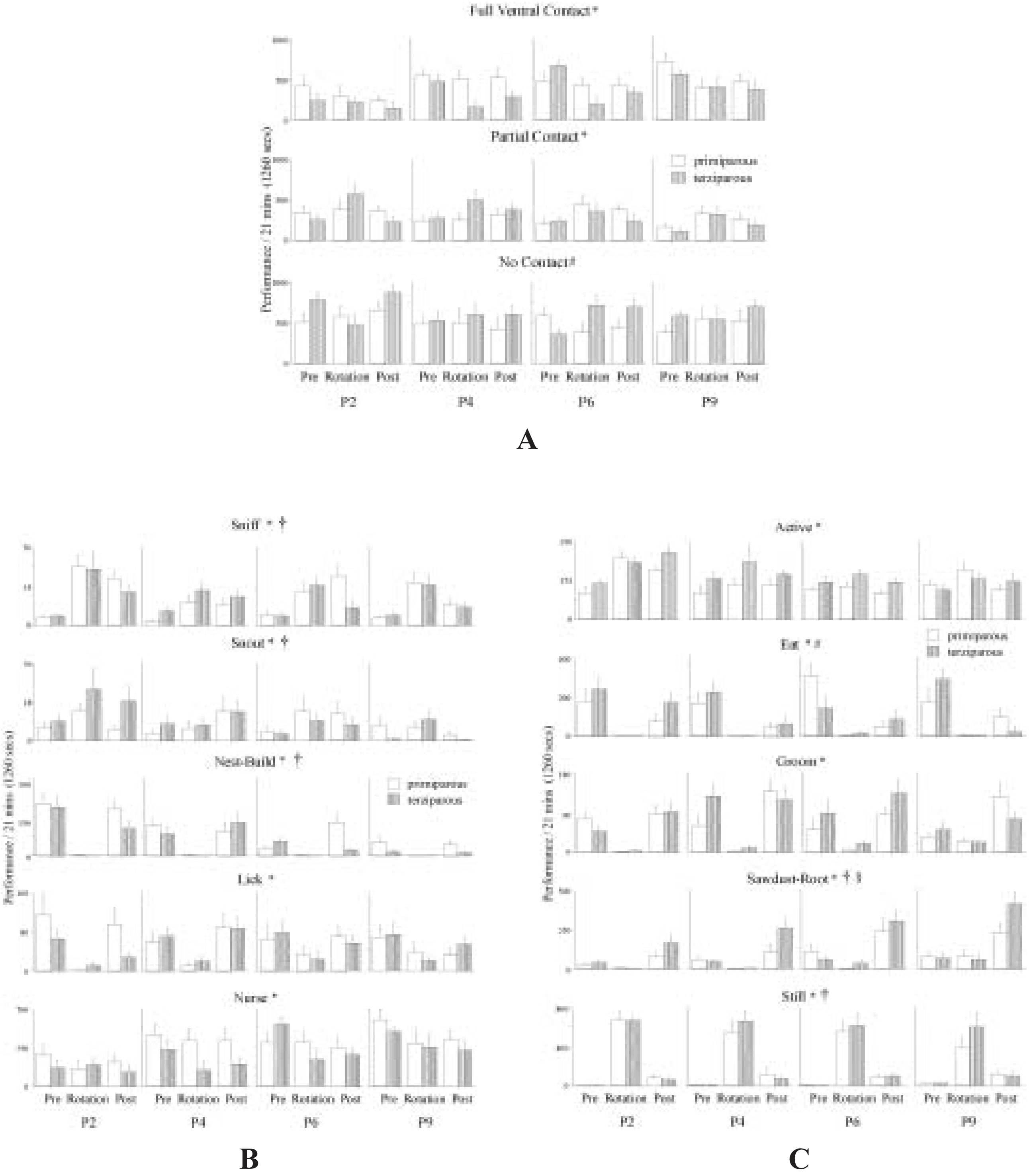

Fig. 1. Behavioral responses (recorded on P2, P4, P6 and P9) of primiparous and terziparous mouse dams exposed daily to hypergravity from P2 to P9. (A) Mother-pup contact; (B, C) behavioral items. Data are means ± SE of total duration of behavior observed in three 7-minute blocks (0–7, 27.5–33.5, 53–60) over the hour before, during and after rotation at 2G, *n*=10. Significant ($P<0.05$) main effects and interactions: (*) phase; (†) day × phase; (§) parity × phase; (#) parity × day × phase, were observed.

Nest = 18.71, 36.64; Nurse = 6.79, 6.36; Active = 9.65, 8.38; Rear = 20.85, 27.89; Sawdust-root = 12.89, 8.46; Still = ns, 4.75, $P<0.05$) (see Fig. 1B,C). Time devoted to sniffing, snouting and nest-building was reduced over days while duration of nursing was progressively extended. Changes in frequencies of these behaviors did not always mirror changes in duration. For example while nursing duration extended steadily over days, frequency rose until P6 but thereafter decreased (data not shown for frequencies).

Active and rearing (data not shown) decreased as days progressed, while sawdust-rooting increased. The duration of Still showed a tendency to decrease as frequency decreased significantly over days.

CHANGES OVER PHASES

Rotational phase significantly influenced all behavioral items measured ($F_{2,36}$ for duration and frequency respectively: Active = 6.80, 4.43; Drink = 25.46, 22.34; Eat = 56.48, 20.44; Groom = 71.41, 39.30; Rear = 16.32, 9.03; Sawdust-root = 37.47, 19.66; Still = 122.24, 29.48; Sniff = 25.35, 6.79; Snout = 3.80, 23.87; Lick = 13.20, 20.70; Nest = 32.14, 40.56; Nurse = 4.84, 4.48, $P<0.05$; data not shown for frequencies). During Rotation sniffing and snouting increased while licking and nest-building were scarcely present. Nursing was also reduced but this effect was comparatively minimal. Active was performed for longer, though less frequently than in Pre-rotation and Still behavior, scarcely present before the rotation, was dramatically increased. Eating and drinking (data not shown) were suppressed almost entirely and grooming was markedly reduced. In Post-rotation behaviors generally tended towards their Pre-rotation baselines. While sniffing, snouting, Still behavior, eating and drinking, only partially recovered in this hour, licking, nest-building, nursing and Active were generally performed at their original levels. Notably, mice performed grooming and sawdust-rooting for longer after Rotation than before, though frequencies tended to resemble Pre-rotation levels.

The influence of rotation varied over days in many cases (day × phase: $F_{6,108}$ for duration and frequency, respectively: Sniff = 2.41, 5.17; Snout = ns, 14.25; Lick = ns, 2.34; Nest = 7.31, 10.31; Nurse = ns, 3.09; Active = 2.64, 3.86; Rear = 10.05, 10.61; Sawdust root = 2.53, 6.38; Still = 3.38, 7.98; Eat = NS, 2.47; Groom = ns, 6.55, $P<0.05$). All the phase-related changes in pup-directed behaviors were particularly accentuated on P2. The suppressive effect of rotation on licking, though not nest-building, lessened over days, a pattern also observed in eating and grooming, at least with respect to frequency. Time spent Still during Rotation gradually diminished over days while Sawdust-rooting in Post-rotation, and to a lesser extent during Rotation, increased over days. Corresponding frequency data indicated that bout lengths of these behaviors were shorter on P2 than on other days. Performance of Active remained elevated after the rotation on P2 but returned to Pre-rotation levels on other days. Performance of rearing was unusually high before and after the rotation on P2 but was otherwise stable over phases.

Effects of parity

A significant main effect of dam parity did not emerge for any of the pup-directed behaviors. However we observed a reduced tendency to nest-build and nurse in terziparous dams ($F_{1,18}=3.83$, $P=0.066$ and $F_{1,18}=3.28$, $P=0.087$, respectively) who spent significantly longer performing Active than their primiparous counterparts ($F_{1,18}=5.52$, $P<0.05$). This disparity in Active levels lessened over days ($F_{3,54}=3.00$, $P<0.05$) and by P9 there was no longer a difference between the two groups.

Differences between parity groups emerged in the Post-rotation phase when terziparous mothers consistently performed more sawdust-rooting (parity × phase $F_{2,36}$: duration = 4.28, frequency = 4.59, $P<0.05$) and primiparous spent longer nest-building (parity × day × phase: $F_{6,108}=2.40$, $P<0.05$), particularly on P2 when they similarly over-expressed licking and on P6 when they also sniffed pups at elevated levels. Terziparous dams showed Post-rotational over-expression of snouting on P2.

Curiously, parity had a day-dependent effect on the frequency of eating bouts ($F_{3,54}=5.08$, $P<0.05$), terziparous dams eating more frequently on P2 than on other days while primiparous mothers ate more frequently on the last two days of observation.

A number of behavioral items (Retrieve, Bar-hang, Dig, Sleep, and Tail-chase) followed a random and sparse pattern across parity status, days and phases and we will not consider them here (results not shown).

Body weights and pup USVs

Both rotated and control groups of primiparous dams gained weight over experimental days ($F_{7,119}$=6.31, P<0.05), however rotated animals were lighter than controls from the first day of rotation until P9 ($F_{1,17}$=20.85, P<0.05) (results not shown). By P21 there was no difference between groups.

All pups also gained weight steadily over the experimental period ($F_{7,119}$=2299.26, P<0.05). Rotated pups were significantly lighter than control pups ($F_{1,17}$=11.34, P<0.05), the difference emerging over days ($F_{7,119}$=19.23, P<0.05) being evident from P3 until P21. Differences in pup weights had disappeared by P33. As expected, we observed sex differences ($F_{1,17}$=35.61, P<0.05) that augmented over days as male pups of both groups gained more weight than females ($F_{7,182}$=4.80, P<0.05).

Finally, USV rate was significantly influenced by postnatal day ($F_{2,180}$=6.52, P<0.05, Fig. 2), vocalizations increasing in frequency between P2 and P5 and thereafter decreasing. Rotated and control pups displayed significantly different USV profiles over experimental days (rotation × day: $F_{2,18}$=4.31, P<0.05) the two groups diverging after P5 when rotated pups failed to show the characteristic decline in USV rate that we observed in controls (see *post-hoc* in Fig. 2).

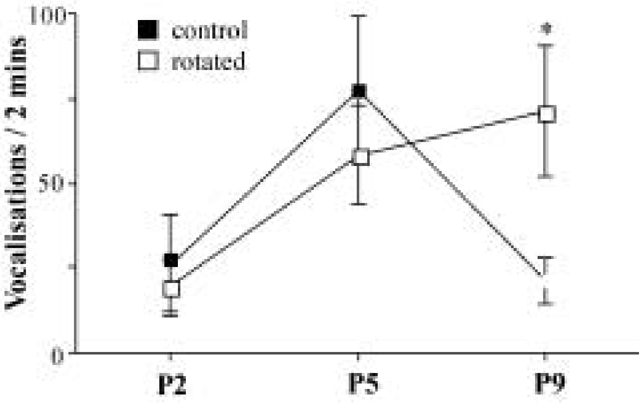

Fig. 2. Number of USVs emitted by pups in 2-minute test one hour following exposure to one hour of 2G. Data are mean ± SE of six litters (one male and one female pup per litter) for each group. * P<0.05.

DISCUSSION

The behavior of the mother mouse is dramatically modified upon rotation at 2G, however nursing behavior and time spent with the pups seem relatively resilient to change. Dams spent a large proportion of the time immobile, confirming reports of a reduction in spontaneous activity in hypergravity (Ronca et al. 2000, Santucci et al. 2000), and movement was largely confined to the performance of gross locomotory or exploratory behaviors. Previous studies have reported several behavioral alterations in rat dams subjected to gravitational changes during pregnancy or lactation including changes in locomotion (Lam et al. 1999), labour contractions (Mills et al. 2000, Ronca and Alberts 2000) and pup-directed behaviors (Megory and Oyama 1984, Mills et al. 2000). In particular, Ronca and coauthors (2001) reported that maternal reproductive experience afforded protection against neonatal mortality in rats exposed to hypergravity from gestational day 11 either throughout birth or throughout the first postnatal week. In the present study mothers sniffed and snouted their pups more than usual but performed almost no licking or nest-building. Though nursing was reduced in some cases, overall it was relatively stable. This is in contrast with the findings of Mills and coauthors (2000) who observed no changes in licking but increased nursing in lactating rat dams exposed to continuous 1.5G centrifugation and Megory and Oyama (1984) who noted a failure to nurse in 50% of rat dams continuously centrifuged above 2G.

Although alterations in the maternal repertoire were generally still evident, though less pronounced, in the hour following the end of rotation, licking, nest-building and nursing recovered fully. Interestingly, self-grooming and sawdust-rooting were over-expressed after the rotation with respect to their Pre-rotation baseline. A corresponding tendency to self-groom immediately following rotation has been previously reported in non-maternal rats (McCaffrey 1985) and mice (Santucci et al. 2000), which also ate sawdust at elevated levels following rotation (unpublished data).

The impact of rotation on behavior of the mother mouse changed over days, exploratory behaviors generally diminishing and several behaviors which were initially suppressed during the rotation progressively re-emerging. However, the possibility that repeated exposures to 2G as well as the experimental procedure *per se* have long lasting influence on the behavior of the animals should be also considered.

Small but consistent differences between parity groups were evident, terziparous dams spending more time Active. Primiparous dams, in contrast, tended to spend longer in Full Ventral Contact with their pups and performing pup-directed behaviors, particularly on early postnatal days. This is consistent with previous observations that inexperienced dams tend to spend more time in the nursing position than experienced animals (Laviola et al. 1994), a tendency that has also been noted in rats (Fleming and Sarker 1990). Although overall levels of No Contact were stable across days and phases, terziparous dams were less consistent. Specifically, they reduced time spent away from their pups in response to the rotation on P2 while on other days showed the opposite, or no response.

The diminution, or over-expression, of specific behaviors confirms that there are elements in the maternal repertoire particularly vulnerable to hypergravity. Specifically, licking and nest-building were heavily inhibited during the rotation. Both nest-building (Noirot 1974) and licking (Moore and Chadwick-Dias 1986) are responsive to age-related pup cues that are likely to be altered in the hypergravitational environment. They also require rather fine motor coordination, known to be undermined by the rotational environment (Lackner and DiZio 1998), while ano-genital licking, which involves the recycling of fluids and nutrients in the mother-pup dyad (Gubernick and Alberts 1983), may be further constrained by the repressive effect of rotation on fluid and food intake (see also Yates et al. 1998). Noirot (1965) observed that a given maternal behavior is more likely to be displayed if it has not been recently performed. Ano-genital licking and nest-building may therefore be expected to occur at elevated levels following their rotation-induced suppression. We did not observe this rebound but it would possibly emerge over a longer post-rotational observation period. The relative resilience of nursing may reflect a strong motivation to perform this behavior, although during rotation dams anyway tend to remain immobile in a position similar to that assumed during nursing.

Elevated levels of exploratory-type behaviors such as Active, rearing and sniffing and snouting the pups were performed on P2, suggesting a reaction to the novelty of the treatment, though proximity to parturition has a similar effect on dam locomotory and rearing expression (Fleming and Sarker 1990). Although further experiments would be required to account for the influence of the concomitant variation in pup age, the gradual decrease over days of sniffing and snouting the pups during rotation and the concurrent increase in the frequency of behaviors such as licking the pups, self-grooming and eating suggests that as days progress animals become habituated to the treatment and are more likely to attempt a variety of different behaviors, although the fatigue of compensating for hypergravity may impede extended performance. These findings support those of Megory and Oyama (1984) who reported that rat dams were more successful in rearing pups under high G-loads if they had been pre-exposed to hypergravity. Previous experience of rotation may therefore be an important determinant of the extent to which the maternal behavioral profile is disrupted upon exposure to this treatment.

In terms of their response to rotation, the two parity groups appeared to differently adjust their coping strategies over successive days of exposure. Several studies have observed that previous maternal experience renders a dam's tendency to behave maternally more resilient to exogenous or endogenous challenge (Bridges et al. 1972, Cohen and Bridges 1981, Kinsley and Bridges 1988, Mann and Bridges 1992, Mann et al. 1989). Conversely, in the present study terziparous mothers readily reduced Full Ventral Contact and the correlated nursing posture during and after the rotation, while concurrently increasing Partial Contact. They were also less likely to regain levels of nest-building and licking after the rotation, particularly on P2, when high levels of snouting were instead observed. A general trend for the two groups to converge over time meant that despite initial differences they were indistinguishable by P9. Physiological disparities between primiparous and terziparous animals may in part explain behavioral differences observed between the two groups. Factors related to body-weight, age and previous pregnancies potentially influence the mother-pup relationship, the animal's ability to adapt to operate in hypergravity and its susceptibility to motion sickness.

In rodents, motion sickness resulting from rotationally-generated hypergravity is reported to induce the ingestion of non-nutritive substances (for review see Wade et al. 2000) a behavioral constituent of sawdust-rooting in the present study, which was observed for increasing periods in the Post-rotation phase over experimental days. Still behavior, performed predominantly during the rotation, has also been associated with motion sickness (Santucci et al. 2000). Interestingly, both these behaviors were displayed at more consistently elevated levels in terziparous dams, suggesting that these mothers were either more susceptible to this condition than primiparous animals or more readily used coping strategies.

Results on body-weights are consistent with previous studies (Kinsley and Bridges 1988, Megory and Oyama 1984, Sondag et al. 1997) confirming that rotation is associated with weight loss in adults and impedes weight gain in pups.

Finally, rotational stimuli were further effective in modulating the USV profile of early postnatal pups. Changes in this behavioral response may in turn affect maternal behavior and future studies could usefully be aimed at investigating this interaction.

CONCLUSIONS

In conclusion, acute rotationally-generated hypergravity has a disruptive effect on behavioral performance in lactating mothers which lessens over repeated exposures. Maternal behavior proved remarkably resilient, however, key elements like nursing and time with pups, fluctuating little and other elements rebounding rapidly after exposure. Previous experience in raising pups has a limited effect on the mother's ability to cope with rotation. Primiparous mothers initially performed pup-directed behaviors more consistently, though the two groups were indistinguishable by the end of the experiment. The developmental delay observed in pups exposed to hypergravity implies that rotating pups on early postnatal days has effects which, whether they operate directly or via deficits in maternal behavior, may be long lasting.

This research is mainly focused on setting up an animal model to study the effects of exposure to altered gravitational states with a smooth transfer of this animal model (which is being developed on Earth using centrifuge-induced hypergravity) to studies under conditions of microgravity aboard of the International Space Station (ISS). To this purpose, the identification of ad hoc modifications to be performed in the cage environment in the animal housing facility placed aboard of the ISS (Mice Drawer System, MDS) to facilitate nesting and mother-offspring interaction and reduce neonatal mortality and/or suffering, will be of crucial relevance. To succeed in space experiments, further studies, also including appropriate rotating and non-rotating controls, should be performed to assess vulnerable items in maternal repertoire in hypergravitational studies.

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REFERENCES

Alberts, JR, Ronca AE (1999) Effects of gravity on development: The importance of behaviour. J Gravit Physiol 6: P5–8.

Alleva E, Caprioli A, Laviola G (1989) Litter gender composition affects maternal behaviour of the primiparous mouse dam (Mus musculus). J Comp Psychol 103: 83–87.

Brennan JF, Santucci D, Branchi I, Alleva E (1999) Ultrasonic vocalizations elicit orienting and associative reactions in preweanling mice. Acta Neurobiol Exp (Wars) 59: 23–30.

Bridges RS, Zarrow M, Gandelman R, Denenberg VH (1972) Differences in maternal responsiveness between lactating and sensitized rats. Dev Psychobiol 5: 123–127.

Cohen J, Bridges RS (1981) Retention of maternal behaviour in nulliparous and primiparous rats: Effects of duration of previous maternal experience. J Comp Physiol Psychol 95: 450–459.

Cohen-Salmon C, Carlier M, Roubertoux P (1982) Differences in patterns of pup care in Mus musculus domesticus. II. Effects of parity on eight inbred strains. Biol Behav 7: 337–346.

Fleming AS, Rosenblatt JS (1974) Maternal behavior in the virgin and lactating rat. J Comp Physiol Psychol 86: 957–972.

Fleming AS, Sarker J (1990) Experience-hormone interactions and maternal behavior in rats. Physiol Behav 47: 1165–1173.

Fox WM (1965) Reflex ontogeny and behavioural development of the mouse. Anim Behav 13: 234–341.

Gubernick DJ, Alberts JR (1983) Maternal licking of young: resource exchange and proximate controls. Physiol Behav 31: 593–601.

Ishay J, Barr-Nea L (1977) Effects of hypergravity on rat fertility, pregnancy, parturition and survival. Experientia 33: 244–246.

Kinsley CH, Bridges RS (1988) Parity-associated reductions in behavioural sensitivity to Opiates Biol Reprod 39: 270–278.

Lackner JR, DiZio P (1998) Adaptation in a rotating artificial gravity environment. Brain Res Rev 28: 194–202.

Lam KP, Mills N, Arrington JL, Baer LA, Ronca AE (1999) Video analysis of pregnancy and parturition in rat dams during 1.5-G centrifugation. American Society for Gravitational and Space Biology Bulletin 13: 58.

Laviola G, Petruzzi S, Rankin J, Alleva E (1994) Induction of maternal behavior by mouse neonates: Influence of dam parity and prenatal oxazepam exposure. Pharmacol Biochem Behav 49: 871–876.

Le Bourg E (1999) A review of the effects of microgravity and of hypergravity on aging and longevity. Exp Gerontol 34: 319–336.

Mann PE, Bridges RS (1992) Neural and endocrine sensitivities to opioids decline as a function of multipary in the rat. Brain Res 580: 241–248.

Mann PE, Kinsley CH, Ronsheim PM, Bridges RS (1989) Long-term effects of parity on opioid and nonopioid behavioural and endocrine responses. Pharmacol Biochem Behav 34: 83–88.

McCaffrey RJ (1985) Appropriateness of kaolin consumption as an index of motion sickness in the rat. Physiol Behav 35: 151–156.

Megory E, Oyama J (1984) Hypergravity effects on litter size, nursing activity, prolactin, TSH, T3, and T4 in the rat. Aviat Space Environ Med 55: 29–35.

Mills NA, Lam KP, Baer LA, Ronca AE (2000) Effects of gravity-loading on rat parturition and maternal care. Dev Psychobiol 36: 238.

Moore CL, Chadwick-Dias A (1986) Behavioural responses of infant rats to maternal licking: Variations with age and sex. Dev Psychobiol 19: 427–438.

Noirot E (1965) Changes in responsiveness to young in the adult mouse III. The effect of immediately preceding performances. Behaviour 24: 318–325.

Noirot E (1972) The onset of maternal behaviour in rats, hamsters and mice. In: Advances in the Study of Animal Behaviour (Lehrman DS, Hinde RA, Shaw E, eds.). Academic Press, New York, Vol. 4, p. 107–145.

Noirot E (1974) Nest-building by the virgin female mouse exposed to ultrasound from inaccessible pups. Anim Behav 22: 410–420.

Orpen BG, Fleming AS (1987) Experience with pups sustains maternal responding in postpartum rats. Physiol Behav 40: 47–54.

Oyama J, Platt WT (1967) Reproduction and growth of mice and rats under conditions of simulated increased gravity. Am J Physiol 212: 164–166.

Plaut K, Maple R, Vyas C, Munaim S, Darling A, Casey T, Alberts JR (1999) The effects of spaceflight on mammary metabolism in pregnant rats. Proc Soc Exp Biol Med 222: 85–89.

Richhardt T (1998) Animal deaths turn shuttle into ‘necrolab’. Nature 393: 4.

Ronca AE, Alberts JR (2000) Effects of spaceflight during pregnancy on labor and birth at 1G. J Appl Physiol 89: 849–854.

Ronca AE, Baer LA, Daunton NG, Sajdel-Sulkowska EM, Wade CE (1999) Biparity enhances postnatal survival but not body weight gain of rats gestated, born and reared during 1.5-G centrifugation. American Society for Gravitational and Space Biology Bulletin Bull 13: 50.

Ronca AE, Baer LA, Mills NA, Sajdel-Sulkowska EM, Wade CE (2000) Body mass, food and water intake, and activity of pregnant and lactating rat dams during 1.5-G centrifugation. J Gravit Physiol 7(2): P131–132.

Ronca AE, Baer LA, Daunton NG, Wade CE (2001) Maternal reproductive experience enhances early postnatal outcome following gestation and birth of rats in hypergravity. Biol Reprod 65: 805–813.

Rosenblatt JS, Lehrman DS (1963) Maternal behaviour in the laboratory rat. In: Maternal Behaviour in Mammals (Reingold HL, ed.). Wiley, New York, p. 8–57.

Santucci D, Corazzi G, Francia N, Antonelli A, Aloe L, Alleva E (2000) Neurobehavioural effects of hypergravity conditions in the adult mouse. Neuroreport 11: 1–4.

Sondag HNPM, de Jong HAA, Oosterveld WJ (1997) Altered behaviour in hamsters conceived and born in hypergravity. Brain Res Bull 43: 289–294.

Wade CE, Ortiz RM, Baer LA (2000) Increases in body mass of rats during spaceflight: Models and measurements. Aviat Space Environ Med 71: 26–30.

Yates BJ, Miller AD, Lucot JB (1998) Physiological basis and pharmocology of motion sickness: An update. Brain Res Bull 47: 395–406.

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