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Effects of mild prenatal decompressions on growth and behavior in the rat

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Two experiments report retarded physiological and behavioral development of rats prenatally exposed to a sequence of rapid, brief decompressions to 6,000 ft. In Experiment 1, prenatally treated young gained weight more slowly than controls, acquired climbing skills at a later age, and ambulated less in an open field at 32 days of age. In Experiment 2, open-field data provided evidence for a critical period during the first 12 days of gestation.

Studies employing real or simulated altitudes in excess of 10,000 ft provide most of the evidence for the prenatal influence of low barometric pressure. Nonetheless, the investigation of "mild" decompressions, involving less than 10,000-ft altitude change, seems equally interesting. In addition to those who live in decompressed atmospheres below 10,000 ft, many individuals experience similarly mild decompressions during air travel.

Effects of these mild decompressions are unknown, but high-altitude studies suggest that reduced barometric pressure generally retards both fetal (Chiodi, 1953; Grahn & Kratchman, 1963; Johnson & Roofe, 1965; Lichty, Ting, Burns, & Dyar, 1957; Mazess, 1965; McClung, 1969; Moore & Price, 1948) and newborn (McCullough & Blackman, 1976) development. The present study attempts to extend these findings to prenatally administered, rapid, brief decompressions to 6,000 ft, like those experienced during air travel.

In the first experiment, rats receiving treatments for 20 days of gestation were observed for body-weight gains and various motor skills, including righting, grasping, walking an inclined plane, and climbing. It was expected that the stressor would reduce body weights of pups and retard appearance of these motor skills relative to untreated controls. Though McCullough and Blackman (1976) reported that acute anoxia did not affect emotionality of 53-day-old rats, weanlings in the second experiment were subjected to open-field testing because decompression exposes animals to potential stressors in addition to hypoxia. Experiment 2 also attempted to isolate a critical period during which prenatal stress maximally influenced stressed offsprings' body weights. This was accomplished with two groups that received treatments for either the first or second 10 days of gestation, the initial expectation being that birth weights in particular would be most limited by hypoxia during the second half of gestation, when weight gains are greatest.

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METHOD

Subjects

Female hooded rats from the department breeding colony which lordosed during mounting were left with a male for 3 h. Animals in each experiment were assigned to groups at random with the restriction that groups within each experiment must contain approximately equal numbers of mothers. In Experiment 1, eight pregnancies resulted in the prenatally stressed group and six in the control group. In Experiment 2, five pregnancies resulted in the 20-day group, four in the 10-day-1 group, six in the 10-day-2 group, and six in the control group.

Equipment

Decompressions in both experiments were delivered in a vacuum chamber, with altitude measured by an altimeter. Prior to each daily run, the altimeter was adjusted for daily intervening changes in atmospheric pressure such that each day's altitude change equaled 6,000 ft. Mothers not receiving treatments on any particular day were kept in an anteroom of the vacuum chamber and were subjected to the same stimuli that stressed groups received, except that unstressed animals were kept at 720 ft (ground level).

All mothers and litters were housed in individual cages except for periodic weighing, weekly replacement of cages and cage litter, and daily feeding and filling of water bottles, although the latter did not require handling of animals. Temperature was kept at 72°F for all animals at all times. Pups were sex typed at their last weighing only.

Open-field behavior was observed in a 3-ft-sq area marked off into 6-in. squares. This field was surrounded by 2-ft-high walls, painted white, and lit by overhead fluorescent room lights.

Procedure

Experiment 1. Prenatally stressed animals received seven daily ascents to and descents from a simulated altitude of 6,000 ft. Treatments began during the second 24 h of pregnancy and continued for 20 days. Each ascent or descent lasted 2 min such that the average rate of pressure change was 2,600 ft/min.

The operator attempted to maintain this average rate with the aid of a rate-of-climb meter. Animals remained at 6,000 ft for 14 min following each ascent and rested at 720 ft for 2 min following each descent. Following the seventh descent each day, animals were left alone at 720 ft. After birth, litters were left alone with their mothers except for weighing at birth and at 14 and 28 days postpartum.

The grasping reflex at birth was measured by holding each pup in one hand with the forelimbs hanging down. A thin metal wire was placed on the sole of one front paw and the presence or absence of grasping was noted. The righting reflex was observed daily between birth and 10 days postpartum by placing each pup gently on its back and recording the latency to place all four paws on the ground. Between 10 and 18 days postpartum, at 2-day intervals, all offspring were tested on the inclined plane and the climbing task. All animals were placed facing up a 28-in. wooden alley tilted at 34 deg. Pups were left at the midpoint of this alley until they slipped to the bottom or climbed to the top. For the climbing task, pups were left hanging by their forepaws from the top edge of a thin metal wall until they either fell (about 2 in.) or pulled themselves completely over the top.

Experiment 2. Controls and a prenatal stress group (Group 20-day) were treated identically to the two groups of Experiment 1 except that all stressed animals of Experiment 2 received six daily ascents to and from 6,000 ft. The 10-day-1 group received the same treatments for 10 days beginning during the second 24 h of pregnancy. Group 10-day-2 received identical treatments to Group 10-day-1 except that its treatments began during the 12th day of pregnancy.

All animals were left undisturbed with their mothers except for weighing at birth, 16 days, and 32 days postpartum. At 32 days of age, all animals were placed for 5 min in one corner of the open field. Ambulation was measured by counting the frequency with which an animal placed its two front paws across one of the lines. In addition, the presence or absence of defecation was recorded.

RESULTS

Mean litter sizes varied greatly between litters and in some cases between groups. This variability was attributed to the short time allowed for mating, and, in an effort to increase power of the analyses, variability in measures due to litter-size variability was partialled out by means of analyses of covariance.

Weights

Analyses of covariance were conducted on all weight data because mean pup weights of litters was always negatively correlated with litter size. Separate analyses were performed at each age because of inequalities in variances at the different ages. There were no significant differences in mean birth weights of litters in either experiment. One control litter was eliminated from all further tests in Experiment 1 because it contained only two live pups shortly after birth.

Experiment 1 stressed pups were significantly lighter than controls at 24 days postpartum [$F(1,11) = 5.57$, $p < .05$], and significant treatment differences occurred at 16 days in Experiment 2 [$F(3,16) = 4.92$, $p < .05$]. A Scheffé test on all pairwise comparisons indicated that 16-day-old "20-day treatment" pups were significantly lighter than pups in the other three groups in Experiment 2. Animals in the other three groups did not differ significantly in weight.

The last weight analysis in both experiments was performed on treatments and sexes. One litter was eliminated from all further analyses in Experiment 2 because it could contribute only two pups. There were no significant effects involving treatments at 28 days postpartum in Experiment 1. However, the analysis of covariance on 32-day-old mean pup weights of litters in Experiment 2 yielded a significant Treatments by Sex interaction [$F(3,15) = 3.49$, $p < .05$]. A Scheffé test found that this interaction resulted from the fact that males were significantly heavier than females in all groups except the 20-day group. In fact, "20-day treatment" males were significantly lighter than males or females in all other groups, but only simple sex differences existed in the other groups.

Motor Skills, Experiment 1

Of the four behavioral measures, only the climbing task was affected by the prenatal treatments. Figure 1 shows that the mean proportion of pups successfully climbing in each litter increased more rapidly for controls than for prenatally stressed animals. A repeated-measures analysis of variance on these proportions, after an arcsin transformation, on Days 12, 14, and 16 yielded a significant interaction between treatments and time of measurement [$F(2,28) = 4.61$, $p < .05$].

The differential weight gains at about this time may be related to this behavior by arguing that increasing ability to climb reflects increasing strength or control over the lower extremities. Our unquantified observations were that successful climbers employed their hind feet more often than unsuccessful climbers. In addition, the increasing proportion of body-weight gains due to trunk and limb growth at this age supports this argument, as does a significant correlation between the

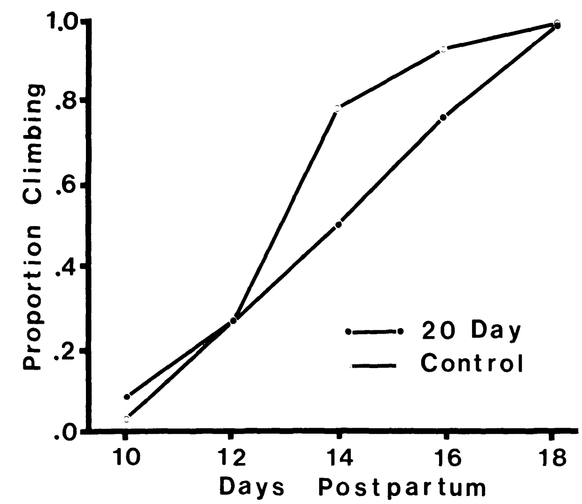

Figure 1. Mean proportion of pups per litter climbing, Experiment 1.

proportion of pups climbing in litters and mean pup weights per litter on Day 14 [$r(14) = .55$, $p < .05$].

Open-Field Test, Experiment 2

Analysis of covariance was conducted on line-crossing data, using litter size as the covariate. A 4 by 2 analysis comparing the four prenatal treatments and activity during the first minute and the last 4 min yielded a significant treatments effect [$F(3,15) = 4.23$, $p < .05$]. A post hoc Scheffé test indicated that this effect resulted from the significantly lower ambulation of 20-day and 10-day-1 litters relative to other animals. Controls did not differ significantly from 10-day-2 animals at any time. Here, too, there is a significant positive correlation between open-field activity and body weights of litters [$r(18) = .38$, $p < .05$].

An analysis of covariance over total defecation by litters in the open field was nonsignificant.

DISCUSSION

In general, prenatal administration of rapid, mild, brief decompressions retards physiological and behavioral development of rats. Body weights reflected this retardation very clearly in both experiments at about 15 days postpartum, but stressed young generally recouped their weight deficit by 30 days of age. The behavior of prenatally stressed offspring in both experiments is best described as less mature than that of controls.

The positive correlations between body weights and performance on the behavioral tasks suggest to us some degree of common causality and that a general debilitation resulted from the prenatal decompressions. Either postnatal maternal variables, such as maternal milk quality (e.g., Tucker, 1964) or maternal interaction with the litter (e.g., Bell, Nitschke, Bell, & Zachman, 1974), or strictly prenatal factors could explain this general debilitation. Decompression and the resultant hypoxia could initiate generalized stress reactions in the mother (Hornbein, 1962), and hormonal consequences of these reactions can cross the placental barrier (Lanman, 1953), although effects of these hormones on offspring behavior are not thoroughly understood at this time (Joffe, Milkovic, & Levine, 1972).

The similar behavior of the "20-day treatment" group and "10-day-1 treatment" group litters of Experiment 2 in the open field implies that the first 12 days of gestation contain a critical period for production of these effects. However, the lack of a similar finding in the weight-gain data suggests that this conclusion be regarded as tentative until future replication.

The inconsistency in the weight analyses between the two experiments at about 30 days postpartum remains unexplained, but we note two known differences in procedures that might explain this discrepancy: (1) Weanlings were weighed at different ages, and (2) Experiment 1 litters were given more decompressions during gestation.

Two practical implications of this work are immediately apparent: (1) If frequent mild decompressions can stress mother rats and their offspring, experiments employing this stressor should be designed using animals that would allow easier extrapolation of results to human populations. (2) Researchers often rely on professional breeders for animal subjects, and these animals are frequently shipped via air carrier. Researchers in the areas of stress and early experience should be aware that this method of shipment may be a source of extraexperimental stress.

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