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Prenatal Stress Reduces Maternal Aggression by Mice Offspring

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POLITCH, J. A. AND L. R. HERRENKOHL. *Prenatal stress reduces maternal aggression by mice offspring.* PHYSIOL. BEHAV. 23(2) 415–418, 1979.—Pregnant mice were subjected to the simultaneous stress of heat, restraint and bright lights during the last trimester of gestation whereas control mothers remained unhandled in the home cage. As adults, prenatally-stressed and nonstressed mice were mated and tested for maternal aggression. Compared with nonstressed controls, prenatally-stressed females bit intruder males on significantly fewer occasions. Prenatal stress therefore reduced maternal aggression by mice offspring. Prenatal stress also significantly reduced maternal and neonatal body weight. Because the appearance of maternal aggression characteristically has been associated with the postpartum period, prenatal stress may reduce maternal aggression in mice by disrupting gonadotropin secretions (possibly prolactin) associated with the lactational phase.

Prenatal stress Maternal aggression Mice

EXPOSING pregnant mothers to adverse environmental stimuli deleteriously affects reproductive functions in female offspring. Overcrowding pregnant mice, for example, markedly reduces sexual responsiveness in female offspring [1]. Moreover, exposing pregnant rats to the simultaneous stress of heat, restraint and bright lights (1) disrupts estrous cycles in female offspring [5]; (2) increases the incidence of high risk pregnancy in offspring through spontaneous abortion and vaginal hemorrhaging [3]; (3) elevates the incidence of stillbirths and neonatal mortality among progeny born to female offspring [3]. Because (1) these latter experiments employed crossfostering [3,5] and (2) prepartal-stress induced alterations in the mothers' behavior and/or lactational performance were not significant [3,5], Herrenkohl [3] has suggested that prenatal stress-induced alterations in later reproductive functions of female offspring are due primarily to alterations in the fetus in utero. Specifically, she has hypothesized that prenatal stress may influence the balance in adrenal and gonadal hormones between mother and fetus or in the fetus alone during a critical hypothalamic differentiation stage to produce gonadotropic hormone deficiencies in adulthood [3]. Serum prolactin levels, for example, have been found to be significantly lower in prenatally-stressed postpartum females that did not maintain litters than in normal lactating rats or in prenatally-stressed females that did maintain young [4].

In mice, the expression of aggression by the female is a behavior pattern that appears to function in the service of the young. The lactating female, particularly early in the postpartum phase, attacks intruder males and wards them off by intense bites directed at the neck or flanks [13]. Although the hormonal basis of maternal aggression is not yet known, there is growing evidence implicating roles for both estrogen and prolactin [13, 14, 16]. The present experiment examines the effects of prenatal stress on the expression of maternal aggression by postpartum female offspring. Because prenatal stress appears to disrupt prolactin-related reproductive activities [4], and because maternal aggression in mice appears to be dependent upon that hormone wholly or in part [13, 14, 16], the possibility arises that prenatal stress may reduce maternal aggression in female offspring, thereby increasing neonatal mortality.

METHOD

Animals and Procedure

Twenty-five female albino CD-1 timed-pregnant mice were obtained from Charles River Laboratories (Wilmington, MA) approximately one week before parturition. They were housed singly under a standard 12 hr light/dark cycle beginning at 8:00 a.m., and maintained on ad lib food and water. On Day 16 of gestation, 12 pregnant females were selected at random and subjected to simultaneous stressors of heat, restraint and bright lights through Day 21 according to methods modified from Ward [15]. Stressed females were placed individually in 12.5×4.5 cm cylindrical Plexiglas restraining cages grouped in a single row under two bright incandescent lights which produced a surface illumination of 475 ft-candles and surface temperature of 33.33°C. Animals were stressed for three 30-min periods per day with 30–45 min periods between the stress periods for rest. Control females were left unhandled in their home cages. At birth, 3 prenatally-stressed and 5 nonstressed litters were randomly selected to provide animals for maternal aggression in adulthood. In addition, to confirm the effectiveness of prenatal stress, 9 prenatally-stressed litters were examined periodically throughout the experiment for reductions in body weight [6]; the 8 remaining nonstressed litters served as body weight controls. At 24 days of age, female offspring were

TABLE 1

EFFECTS OF PRENATAL STRESS ON MATERNAL AGGRESSION AND POSTPARTUM BODY WEIGHT, AND ON NEONATAL BODY WEIGHT AND MORTALITY[1]

	n	Prenatally Stressed Females	n	Nonstressed Females
Maternal Aggression				
Number of bites	10[2]	8.70 ± 2.48*	9[2]	26.67 ± 7.57
Maternal Body Weight				
Postpartum Day 1	19	32.66 ± 0.57§	19	36.17 ± 0.70
Postpartum Day 2	19	33.92 ± 0.50‡	19	37.14 ± 0.81
Postpartum Day 3	19	34.13 ± 0.54‡	19	37.33 ± 0.81
Postpartum Day 7	19	37.60 ± 0.59§	19	41.11 ± 0.72
Neonatal Body Weight[3]				
Postpartum Day 1	19	1.56 ± 0.03*	19	1.64 ± 0.03
Postpartum Day 2	19	1.76 ± 0.05*	19	1.89 ± 0.04
Postpartum Day 3	19	1.93 ± 0.07†	19	2.17 ± 0.07
Postpartum Day 7	19	3.37 ± 0.17*	19	3.86 ± 0.16
Neonatal Mortality[4]	19	1.37 ± 0.38§	19	0.21 ± 0.12

*$p<0.05$
†$p<0.02$
‡$p<0.01$
§$p<0.001$
1. All measures Mean ± standard error.
2. All females fought with males.
3. Per pup per litter, in grams.
4. Number dead pups per litter, postpartum days 1–7.

weaned and housed with female littermates in 24×32×16 cm Fiberglass observation cages provided with San-i-cel bedding. They were fed Purina pellets and watered ad lib in colony rooms maintained at a temperature of 72°F on 12-hr light daily beginning at 8:00 a.m. At 70 days of age, prenatally-stressed and nonstressed females were placed with adult CD-1 male mice for one week for mating. Following removal of males, females were housed individually in the Fiberglass cages and examined daily for the day of birth. On the day of birth, 19 prenatally-stressed and 19 nonstressed dams along with their offspring were selected at random and weighed during the first postpartum week. In addition, pup mortality was recorded.

Aggression Testing

Ten prenatally-stressed and 9 nonstressed females selected at random were left undisturbed for the first postpartum week. On postpartum Day 7, these animals were tested for maternal aggression according to a modification of methods described by Svare [13]. Specifically, tests for aggressiveness were performed by introducing a naive adult CD-1 male mouse into the cage of each female for a period of 5 min. Aggressiveness was assessed by recording the frequency of biting behavior by the experimental female during the test period. All aggression testing took place between 3:00 and 5:00 p.m. Each male intruder was housed in a 24×32×16 cm Fiberglass cage with five other similar males and was used for no more than two aggression tests.

RESULTS

Table 1 shows that prenatally-stressed female offspring bit intruder males on significantly fewer occasions than did nonstressed females ($t=1.36$, $df=17$, $p<0.05$).

Table 1 also shows that prenatal stress significantly depressed the body weights of females and their offspring during the lactation period (i.e., on postpartum Days 1, 2, 3 and 7). The differences in body weight between prenatally-stressed and nonstressed females were highly significant (postpartum Days 1, 2, 3 and 7; respective t's=4.23, 3.35, 3.23 and 3.69; df's=36; p's<0.001, 0.01, 0.01 and 0.001). Differences in prenatally-stressed and nonstressed offspring body weight were also significant (postpartum Days 1, 2, 3 and 7; respective t's=2.08, 2.16, 2.47 and 2.04; df's=36; p's<0.05, 0.05, 0.02 and 0.05). Moreover, during the first postpartum week, neonatal mortalities were significantly higher in the prenatal stress condition than in the nonstressed group $t(36)=53.66$, $p<0.001$).

Additional observations revealed that differences in body weights between the prenatal stress and control conditions were apparent even before the postpartum phase. At 45 days of age, for example, analysis of the mean body weights of each litter showed that the prenatally-stressed animals weighed less than nonstressed controls ($mean_{PS}=21.33 \pm 0.38$, $mean_{NS}=23.19 \pm 0.46$; $t(15)=3.17$, $p<0.01$). Similarly, at 70 days of age (just prior to mating) prenatally-stressed females weighed less than nonstressed controls ($mean_{PS}=25.5 \pm 0.49$, $mean_{NS}=28.3 \pm 0.51$; $t(38)=4.00$, $p<0.001$).

DISCUSSION

The present experiment demonstrates that prenatal stress decreases not only the amount of maternal aggression dis-

played by female offspring but also reduces maternal and offspring body weights and increases neonatal mortality. The mechanism whereby prenatal stress disrupts these reproductive functions in female offspring is not yet known. Because crossfostering was not employed in the present experiment, one possibility is that prepartal stress-induced disturbances in the mothers' behavior and/or lactation may be the primary cause of postpartum disorders in female offspring. This possibility appears unlikely, however, because in a variety of prenatal stress experiments employing crossfostering, neither of these conditions were found to significantly alter dependent variables as diverse as (1) litter weight of both sexes during the postpartum period and adult sexual behavior of male offspring [6], (2) the prolactin and corticosterone response to ether stress in prenatally-stressed male rats as adults [11], (3) estrous cycling in female offspring [5], and (4) the maintenance of pregnancy and progeny by female offspring during the gestational and postpartum phase [3]. Prepartally-stressed mothers did not differ significantly from prepartally-nonstressed mothers with respect to the latency of litter retrievals or the duration of nursing behavior regardless of whether they were rearing prenatally-stressed or prenatally-nonstressed offspring [6]. Prenatal stress, therefore, appears to exert its primary action on later reproductive capabilities in offspring by alterations during the in utero stage, possibly by altering the hormonal milieu.

The major finding of the present experiment was that prenatal stress decreased the amount of maternal aggressive behavior displayed by female offspring. There are several explanations for this finding. First, stress has been shown to increase the levels of several hormones including adrenocorticotropic hormone (ACTH) and corticosterone [7,17]. It is possible that increased levels of either or both of these hormones during the prenatal period may have an effect on the long-term development of the neurohormonal mechanisms that control maternal aggression. In support of this possibility are the recent observations of Simon and Gandelman [12] that offspring of female mice treated with ACTH during the last trimester of pregnancy showed decreased aggressive behavior in adulthood. These authors postulated that corticosterone (stimulated by the high levels of ACTH) may in fact be the crucial hormone for this effect.

Another possibility is that prenatal stress may reduce maternal aggression by reducing lactogenic hormones more directly. In most cases, maternal aggression is uniquely associated with the lactational condition in the female [13]. Prenatal stressors similar to those employed in the present experiment have already been shown to (1) markedly alter concentrations of the neurotransmitter dopamine in the hypothalamic arcuate nucleus of female rat offspring [9]; and (2) increase the incidence of mortality among neonates born to prenatally-stressed female rat offspring [3]. Because marked alterations in arcuate dopamine have been associated with abnormalities in gonadotropic hormone and prolactin release from the anterior pituitary gland [2, 8, 10] and because lactational disorders appear to be the primary cause of neonatal mortality among offspring of prenatally-stressed females [3], it is possible that one means by which prenatal stress may affect later reproductive-related functions in mice is by reducing lactation-related activities (i.e., gonadotropic hormones or prolactin specifically).

In addition to the effects on maternal aggression, prenatal stress significantly reduced maternal and offspring body weights and significantly increased neonatal mortality (Table 1). All of these actions are suggestive of, but do not conclusively prove, the existence of lactational difficulties. However, there is evidence that prenatal stress significantly reduces the elevation of serum prolactin shown by males to the introduction of ether stress in adulthood [11]. Moreover, Wise and Pryor [16] have already shown that prolactin is important for the occurrence of maternal aggression in hamsters. The possibility that prenatal stress may decrease maternal aggression in mice by affecting prolactin is presently being explored.

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REFERENCES

1. Allen, T. O. and B. N. Haggert. Group housing of pregnant mice reduces copulatory receptivity of female progeny. *Physiol. Behav.* **19**: 61–68, 1977.
2. Barraclough, C. A., J. L. Turgeon and O. M. Cramer. Neural correlates of adenohypophyseal LH release in rats. In: *Anatomical Neuroendocrinology*, edited by W. E. Stumpf and L. D. Grant. New York: Karger, 1975, pp. 200–207.
3. Herrenkohl, L. R. Prenatal stress reduces fertility and fecundity in female offspring. Submitted to *Science*, 1978; (Also Paper Read at the 86th Annual Convention of the American Psychological Assoc., Toronto, Canada, 1978).
4. Herrenkohl, L. R. and R. R. Gala. Serum prolactin levels and maintenance of progeny by prenatally-stressed female offspring. *Experientia*, in press, 1979.
5. Herrenkohl, L. R. and J. A. Politch. Effects of prenatal stress on the estrous cycle of female offspring and adults. *Experientia* **34**: 1240–1241, 1978.
6. Herrenkohl, L. R. and J. B. Whitney. Effects of prepartal stress on postpartal nursing behavior, litter development and adult sexual behavior. *Physiol. Behav.* **17**: 1019–1021, 1976.
7. Knigge, K. M. and R. M. Hoar. A procedure for measuring plasma binding of adrenal corticoids: effects of acute and chronic stress. *Proc. Soc. exp. Biol. Med.* **113**: 623–627, 1963.
8. MacLeod, R. M. Regulation of pituitary function by catecholamines. In: *Mammary Cancer and Neuroendocrine Therapy*, edited by B. A. Stoll. London: Butterworths, 1974, pp. 139–159.
9. Moyer, J. A., L. R. Herrenkohl and D. M. Jacobowitz. Stress during pregnancy: effects on concentrations of discrete brain region catecholamines in offspring as adults. *Brain Res.* **144**: 173–178, 1978.
10. Neill, J. D. Prolactin: its secretion and control. In: *Handbook of Physiology-Endocrinology*, Vol. IV, edited by E. Knobil and W. H. Sawyer. Baltimore: Waverly, 1974, pp. 469–488.

11. Politch, J. A., L. R. Herrenkohl and R. R. Gala. Effects of ether stress on prolactin and corticosterone levels in prenatally-stressed male rats as adults. *Physiol. Behav.* **20:** 91–93, 1978.
12. Simon, N. G. and R. Gandelman. Decreased aggressive behavior in the offspring of ACTH-treated mice. *Behav. Biol.* **21:** 478–488, 1977.
13. Svare, B. B. Maternal aggression in mice: influence of the young. *Biobehav. Rev.* **1:** 151–164, 1977.
14. Svare, B. and R. Gandelman. Postpartum aggression in mice: Inhibitory effect of estrogen. *Physiol. Behav.* **14:** 31–35, 1975.
15. Ward, I. L. Prenatal stress feminizes and demasculinizes the behavior of males. *Science* **175:** 82–84, 1972.
16. Wise, D. A. and T. L. Pryor. Effects of ergocornine and prolactin on aggression in the postpartum golden hamster. *Hormones Behav.* **8:** 30–39, 1977.
17. Yasuda, N., K. Takeve and M. A. Greer. Evidence of nycterohemeral periodicity in stress-induced pituitary-adrenal activation. *Neuroendocrinology* **21:** 214–224, 1976.