Physiology & Behavior, Vol. 32, pp. 95–99. Copyright © Pergamon Press Ltd., 1984. Printed in the U.S.A. 0031-9384/84 $3.00 + .00

Effects of Prenatal Stress on Reproduction in Male and Female Mice

JOSEPH A. POLITCH[1] AND LORRAINE ROTH HERRENKOHL[2]

Psychology Department, Temple University, Philadelphia, PA 19122

Received 23 June 1983

POLITCH, J. A. AND L. R. HERRENKOHL. *Effects of prenatal stress on reproduction in male and female mice.* PHYSIOL BEHAV 32(1) 95–99, 1984.—Pregnant mice were exposed to heat-restraint stress from Days 14 through 21 of gestation. Feminine receptivity quotients were significantly higher in prenatally-stressed male offspring than in unhandled males; however there were no differences in testes weights or masculine copulatory behavior. Prenatally stressed females exhibited vaginal opening at a later date, had longer estrus cycles and higher median quality receptivity scores than unhandled controls. Prenatal stress had no profound effects on pregnancy, parturition or survival of young. However there was a significantly smaller proportion of parturient postnatally stressed females compared to unhandled controls.

Prenatal stress Reproduction Male and female mice

WARD [16,18] has described the prenatal stress syndrome in male rats as being characterized by demasculinization and feminization of sexual behavior. She exposed late pregnant rats to heat-restraint stress during the last trimester of gestation and reported that male offspring as adults exhibited reductions in copulatory behavior and increases in lordotic potential. Although Rhees and Fleming [13] have replicated these results and extended the findings to nutritional stress and injections of ACTH, the prenatal stress literature has not been without its inconsistencies [3]. Our laboratory, for example [19], and that of Larsson [4] have been able to reproduce only the feminizing action of heat-restraint in prenatally stressed rats, and not the demasculinizing action. Such differences may be attributed to differences in strain of animal, housing or stress procedures [2,5].

Inconsistencies also exist in the literature on prenatal stress effects on female offspring. Herrenkohl [6] exposed late pregnant rats to heat-restraint stress and described a syndrome in female offspring marked by reproductive deficiencies in adulthood (estrus cycle disorders, spontaneous abortions and vaginal hemorrhaging during pregnancy, stillbirths, neonatal mortality and low birth-weight young). Ward [17] found that prenatal stress did not alter sexual behavior of female offspring. Beckhardt and Ward [2] employed the same heat-restraint stress that demasculinized and feminized sexual behavior of male offspring and reported that reproductive capabilities of female offspring remained unimpaired. Differences in findings between the laboratories of Herrenkohl [6,7] and Ward [2,17] could be due to differences in stress procedures [2].

An even more intriguing possibility is that there is a sex difference in the amount of stress needed to disrupt adult reproductive functioning. In other words, the intensity of stressor required to modify sexual behavior of male offspring may differ from that required to alter reproductive capabilities of female offspring. In all of the stress research, no one report has examined the influence of prenatal stress on reproductive capabilities of littermates of both sexes. Nor has prenatal heat-restraint stress been applied to examine reproductive capabilities in adulthood of species other than rats. Prenatal restraint-heat stress has been found to reduce the maternal aggression in mice characteristically shown toward male intruders by female progeny during the postpartum phase [12]. Another kind of prenatal environmental stress, namely overcrowding, has been shown to influence reproduction in adult mice: group housing of pregnant mice reduced copulatory receptivity of female offspring [1]. The present experiment was conducted to examine the effects of prenatal restraint-heat stress on reproductive capabilities of male and female littermates in adulthood. By way of attempting to generalize prenatal stress findings to other species, mice instead of rats became the subjects of choice.

METHOD

Animals and Procedure

Twenty-four female albino CD-1 mice, bred from stock obtained from Charles River Laboratories (Wilmington, MA), were time mated at 75 days of age. Following insemination, animals were housed singly in 32×29×14.5 cm fiberglass cages under a standard 12 hr light/dark cycle beginning at 8:00 a.m. EST, and maintained on ad lib food and water. On Day 14 of gestation, 7 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 [16] and previously described by Herrenkohl [6] and Herrenkohl and Politch [7]. Prenatally

[1]Current address: Department of Neuroscience, Children's Hospital Medical Center, 300 Longwood Avenue, Boston, MA 02115.
[2]Requests for reprints should be addressed to Lorraine Herrenkohl, Psychology Department, Temple University, Philadelphia, PA 19122.

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-min periods between the stress periods for rest. The remaining pregnant females were allowed to give birth without prenatal manipulation. Eight litters were randomly selected and submitted to postnatal stress to control for the stage of development in which stress was introduced. Individual litters were separated from their mothers, placed in the cylindrical restraining cages, and subjected to the stress regimen described above from the day of birth (Postpartum Day 0) through Postpartum Day 3. Offspring of the unhandled females served as controls. To reduce extraneous stress, offspring remained with their biological mothers. Previous experiments with prenatal stress in rats had indicated that postnatal maternal conditions did not contribute to reproductive deficiencies of offspring in adulthood [6,8]. To avoid litter effects, there was equal representation of at least 5 different litters within each condition.

All litters were weaned in fiberglass cages on Day 21. Offspring of the same sex were housed in 24.5×18×18 cm metal hanging cages (Hardco Scientific) on Day 28. Subjects were placed under reversed lighting conditions on Day 60 so that light onset occurred at 8:00 p.m. EST.

Male Offspring

On Day 80, all males were bilaterally castrated under sodium pentobarbital anesthesia (Nembutal Sodium) and testes were weighed. Following surgery, subjects were housed individually in hanging cages and were administered daily injections of testosterone propionate (150 μg/0.05 ml peanut oil). On Day 95, tests for masculine copulatory behavior commenced with methods modified from Allen and Haggett [1] and McGill [9,10]. Tests began 4 hr after the start of the dark phase of the light/dark cycle and 3 hr following TP injection. Female lures had been ovariectomized previously and administered an intramuscular injection of estradiol benzoate (EB) (80 μg/0.05 ml peanut oil) on 2 successive days. Twenty-four hr after the last EB injection, females were given an IM injection of progesterone (P) (500 μg/0.05 ml peanut oil). Six hr following the P injection, females were screened for sexual receptivity by exposing each lure to a vigorous stud male; only females who received a receptivity score of 2.0 or higher out of 3 (see *Female Offspring*, below) were used as lures. Tests for male copulatory behavior began immediately after screening by introducing the female into the cage of the male. A sheet of Plexiglas was placed over the top of the cage to facilitate observation. During the behavior test, the following measures of male copulatory behavior were taken [9,10]: Mount Latency, Intromission Latency and Ejaculation Latency. If the experimental male did not intromit within the first 20 min, the test was terminated. If the male did intromit within the first 20 min of the encounter, the test was extended until ejaculation or until 60 min had elapsed. All animals were given three behavior tests at least 1 week apart or were tested to ejaculation, whichever came first.

No animal was excluded from the analysis. If a subject ejaculated on the first test, mount latency and intromission latency were derived from that test. If the subject ejaculated on the second test, mount latency and intromission latency were the mean scores of the two tests. If the subject ejaculated on the third test (or not at all), mount latency and intromission latency were the median scores of the three tests. If a subject did not mount or intromit in any test, he was given a maximum score. There were no significant differences amoung the groups in tests to ejaculation. The means and standard errors for the Prenatal Stress, Postnatal Stress and Unhandled groups were 2.78±0.31, 3.07±0.33 and 3.46±0.18 respectively, $F(2,43)=1.32$, $p>0.05$.

After the criterion for masculine copulatory behavior was met, daily TP injections were terminated. At least 3 weeks following male behavior testing, tests for feminine copulatory behavior were begun. Tests for female behavior were conducted in a 30×13.5×30 cm plywood box with a Plexiglas front panel. The interior of the chamber was coated with polyurethane to minimize the absorption of urine and feces. On Day 140 of life, males were given the same hormone regimen previously described for female lures. Approximately 6 hr following the P injection, experimental males were placed into the test arena which contained a previously screened vigorous stud male who had been given a 15 min acclimation period in the test chamber. Subjects were tested for feminine copulatory behavior for 90 min or until they had received 15 mounts, whichever came first.

Receptivity to each mount was rated on a 3-point scale [9,10]: "1"=a complete lack of receptivity which included active avoidance of the stude male; "2"=average receptivity with some squeaking and movement during the mount, and the mount was terminated by the experimental animal; "3"=good to high receptivity which featured a minimum of squeaking and movement by the experimental male, and the mount was terminated by the stud male. Three measures of feminine copulatory behavior were derived from each subject's scores in each test as described previously [1]: the median score, the maximum response (the highest score displayed by the subject in a given test), and the receptivity quotient [RQ=number of receptive responses (scores of 2 or 3)/number of mounts ×100]. Subjects were given two tests of feminine copulatory behavior, the second occurring 7 days following the first.

Female Offspring

Beginning on Day 30, all females were examined for vaginal opening daily. Commencing on Day 60, females were checked for stage of the estrus cycle by vaginal lavage for 20 consecutive days. On Day 80, subjects were placed under reversed lighting conditions so that light onset occurred at 8:00 p.m. EST. On Day 120, the taking of daily estrus smears was resumed, 2 hr after the onset of the dark phase of the light/dark cycle. If females were found to be in estrus (indicated by a smear consisting primarily of cornified cells), they were tested for feminine copulatory behavior. Tests for female behavior were conducted in the plywood test chamber. Testing commenced approximately 2 hr after vaginal lavage by placing a proven stud male CD-1 conspecific in a clean test chamber for a minimum of 15 min for acclimation. An experimental female was then placed in the chamber and tests lasted until the subject had received 25 mounts or until the male ejaculated.

Receptivity of the female to each mount was rated on the 3-point scale previously described [9,10] and three measures of feminine copulatory behavior were derived from each subject's scores in each test [1]. Females were tested until they were inseminated or until they had received 5 behavioral tests (one test was administered on each day the female was

TABLE 1

EFFECTS OF PRENATAL STRESS ON TESTICULAR DEVELOPMENT AND REPRODUCTIVE BEHAVIOR IN MALE MICE

			Measures							
			Masculine Copulatory Behaviors				Feminine Copulatory Behaviors*			
	N	Testes Weight	N	Mount Latency (Median, in sec)	Intromission Latency (Median, in sec)	Ejaculatory Latency (sec)	N	Median Response	Maximum Response	Receptivity Quotient
Prenatal Stess	21	0.31±0.01	18	740.83± 94.06	907.94± 82.94	2275.83±324.71	19	1.37±0.19†	1.84±0.23†	38.60±9.71†‡
Postnatal Stress	15	0.30±0.01	15	650.07± 96.14	992.73± 82.39	2896.67±289.92	15	0.57±0.18	0.73±0.23	13.11±7.31
Unhandled	14	0.29±0.01	13	747.92±114.87	909.62±107.52	2731.54±306.53	11	0.91±0.16	1.18±0.23	11.21±6.39

*Results of Test 1: Test 2 findings were similar.
†Prenatal Stress group differed significantly from Postnatal Stress, $p<0.05$, Duncan's Multiple Range test.
‡Prenatal Stress group differed significantly from the Unhandled group, $p<0.05$, Duncan's Multiple Range test.

TABLE 2

EFFECTS OF PRENATAL STRESS ON PRODUCTIVE CAPABILITIES IN FEMALE MICE

						Measures*					
						Lordosis			Pregnancy	Parturition	Postpartum
						Median					
Treatment	N	Day of Vaginal Opening	N	Length of Estrus Cycle (Median, in days)	N	Receptivity Score	Receptivity Quotient	N	Proportion of Females Inseminated	Proportion of Females Giving Birth	Proportion of Litters Maintained
Prenatal Stress	19	33.58±0.37†	19	6.42±0.37†	19	1.39±0.13†	30.38±8.38	19	16/19	12/19	12/19
Postnatal Stress	19	32.32±0.42	18	5.50±0.35	18	1.06±0.06	11.11±3.41	18	14/18	7/18†	7/18†
Unhandled	19	32.37±0.33	17	4.91±0.25	16	1.16±0.09	21.36±7.43	17	14/17	14/17	14/17

*Means ± standard errors.
†Prenatal Stress group significantly differs from Unhandled group, $p<0.05$, Duncan's Multiple Range test.
‡Postnatal Stress group significantly differs from Unhandled group, $p<0.01$, χ^2 test.

in estrus), whichever came first. If females were not inseminated after receiving five tests, they were placed (while in estrus) with a stud male for approximately 15 hr. Animals were weighed periodically throughout the course of the study. These methods were modified from those reported by Turner and Taylor [15] in female rats.

After the criterion described above was met, animals were housed singly in fiberglass cages, vaginal lavage was stopped, and females were given strips of paper for nest building. Females were examined daily to determine gestation and the onset of delivery. Immediately following birth, litters were examined, weighed and observed until Postpartum Day 21. The proportion of females inseminated that gave birth was recorded, as was the proportion of litters that was maintained.

Continuous data were analyzed by one-way analyses of variance. Significant main effects were followed by Duncan's multiple range tests at the 0.05 level of significance. Frequency data were analyzed by Chi^2 tests.

RESULTS

Table 1 summarizes the means±standard errors of testes weights and copulatory behaviors in male offspring. There were no significant differences among groups in testes weights or masculine copulatory behavior when scores were analyzed for each individual behavior test or when median scores were utilized, F's(2,44–48)=3.23, $p>0.05$. As a result, only the most important variables were listed in Table 1.

With respect to feminine copulatory behaviors shown by the males, one-way analyses of variance indicated a significant main effect for prenatal stress for the median response, $F(2,41)=5.29$, $p<0.01$, the maximum response, $F(2,41)=6.31$, $p<0.01$, and the receptivity quotient, $F(2,41)=3.33$, $p<0.05$, in Test 1. The Prenatal Stress group had higher values than all other groups for all three variables. However post hoc analysis revealed that the Prenatal Stress group differed from the Postnatal Stress and Unhandled groups for the measure of receptivity quotient only. For the measures of median response and maximum response, the Prenatal Stress group differed significantly from the Post natal Stress group but not from the Unhandled group. The Postnatal Stress group did not differ from the Unhandled group on any of the measures of feminine copulatory behavior in Test 1. Although the Prenatal Stress group continued to have higher values for all feminine copulatory behavior

variables in Test 2, none of the differences reached statistical significance, F's(2,41)=3.23, $p>0.05$.

Table 2 summarizes the effects of prenatal stress on reproductive capabilities of female offspring. One-way analyses of variance revealed a significant main effect for prenatal stress for the variables of day of vaginal opening, $F(2,54)=3.66$, $p<0.05$, median length of estrus cycle, $F(2,51)=5.3$, $p<0.01$, and median quality score of feminine receptivity (over the entire number of tests to a maximum of five tests), $F(2,50)=3.27$, $p<0.05$. Post hoc analysis revealed that the Prenatal Stress group exhibited vaginal opening at a later age, had longer estrus cycles, and had higher median quality receptivity scores than Unhandled subjects, whereas the Postnatal Stress group did not differ from Unhandled controls on these measures. Although the Prenatal Stress group had a higher median receptivity quotient than the other groups, the differences were not significant, $F(2,50)=2.10$, $p>0.05$. An overall Chi^2 test showed that the proportion of animals inseminated did not differ among the three groups, $\chi^2(2)=0.27$, $p>0.05$. Another overall Chi^2 test indicated that there was a significant difference in the proportion of animals that gave birth following insemination or after being housed with a male, $\chi^2(2)=6.99$, $p<0.05$. Individual comparisons of the Prenatal and Postnatal Stress groups with the Unhandled controls indicated that the Postnatal Stress group had a significantly smaller proportion of parturient animals in comparison to the Unhandled controls, $\chi^2(1)=6.89$, p's<0.01. The Prenatal Stress group did not differ from Unhandled controls on this measure, $\chi^2(1)=1.65$, $p>0.05$. Autopsies revealed no sign of implantation sites or fetal resorption in females that did not give birth. All the mothers who gave birth maintained their litters. There were no other significant differences among the groups on reproductive indices.

DISCUSSION

Prenatal stress had no effect on testes weights or masculine copulatory behavior of male mice; however, it did increase some dimensions of feminine copulatory behavior. Feminine receptivity quotients were significantly higher in prenatally-stressed male mice than in unhandled ones (Table 1). Such findings on the sexual behavior of male mice differ in part from those of Ward [16,18] and Rhees and Fleming [13] who reported that heat-restraint stress both demasculinized and feminized sexual behavior of male rats. However, the findings are consistent with reports of Whitney and Herrenkohl [19] and Dählof *et al.* [4], which employed immobilization-illumination stress, and found feminization, but not demasculinization, of sexual behavior in male rats.

In an investigation of the effects of prenatal stress on sexual behavior, Whitney and Herrenkohl [19] reported that anterior hypothalamic lesions that disrupted feminine, but not masculine, sexual behavior of female rats also reduced the feminized sexual behavior of prenatally stressed male rats. In that experiment, which employed heat-restraint stress of twice the illumination of Ward [16,18], copulatory behavior of all subjects was normal including that of sham-lesioned prenatally stressed males. Thus prenatal stress feminized but did not demasculinize sexual behavior of male rats. Dählof *et al.* [4] exposed pregnant rats to crowded living conditions, or to immobilization-illumination stress, and found that male offspring in adulthood showed increased readiness to exhibit lordosis while no deficits were observed in masculine sexual behavior. Differences in stress procedures may be invoked as an explanation for discrepancies in results. Dunlap *et al.* [5] suggest that one other factor that contributes to the prenatal stress effect is group housing. She and co-workers were able to demonstrate that isolation enhanced the demasculinizing action of stress and that therapy with a young female improved the performance of all males except those subjected to both stress and isolation.

Prenatal stress delayed vaginal opening and increased estrus cycle length in female mice (Table 2). Similar findings were reported by Paris and Ramaley [11] upon exposure of pregnant and neonatal mice to heat stress. However prenatal stress did not lead to the profound disturbances in pregnancy, parturition or survival of young that Paris and Ramaley [11] reported in mice and that Herrenkohl [6] found in rats.

Beckhardt and Ward [2] reported the reproductive functioning was unimpaired in prenatally stressed female rats. They restrained rat mothers under bright, hot lights from Days 14 through 21 of gestation and found that female offspring had normal cyclicity, sexual behavior, pregnancy, parturition, pup survival and maternal behavior. In an attempt to explain differences in findings between Herrenkohl's laboratory [6,7] and their own [2], the authors invoked differences in stress procedures. Compared to Ward [2, 16, 17, 18], Herrenkohl [6] and Herrenkohl and Politch [7] stressed rats one day earlier and one day longer during gestation, during the light phase of the diurnal cycle, and used mothers that were shipped while pregnant from the supplier, thus incurring another possible stress [14]. In addition, Herrenkohl's stress procedure [6, 7, 19] involved twice the illumination of Ward [2, 16, 18]. It is possible that differences in the intensity of the stress utilized in the present study in mice and by Herrenkohl [6] in rats may explain differences in findings between the two experiments. It is also possible that there is a species-specific responsiveness to stress.

The final findings (and ones of some curiosity) include the observation that prenatal stress seemed to "enhance" sexual behavior in female mice. There was a significant *increase* in median quality score of feminine receptivity in stressed mice compared to controls (Table 2). This finding differs from that of Allen and Haggett[1] who found that prenatal crowding reduced sexual receptivity in female mice. We are unable to explain these findings at the present time.

There was no evidence for a sex difference in responsivity to prenatal stress in the present research. The reproductive capabilities of both sexes were affected in adulthood by heat-restraint applied to dams during the last trimester of gestation. In any case the present findings on prenatal stress applied to mice differ from those in rats [2, 4, 5, 6, 7, 13, 16, 18, 19]. Whether the partial feminization of sexual behavior in male mice and the partial enhancement of feminization in female mice are due to some specific actions associated with a particular kind of stress, or to species-specific responsivity, remains to be pursued. It should also be noted that some effects of postnatal stress were detected: when compared with prenatally stressed and unhandled mice, heat-restraint applied to neonatal female mice significantly reduced the incidence of births (Table 1). Perinatal stress may have differential effects on reproductive capabilities depending upon the organism, the maturational stage of the reproductive characteristic and the nature of the stress.

ACKNOWLEDGEMENTS

Support for the research was provided by Research Support Grant 5-S07-RR07115-12 from the Biomedical Research Program of the Department of Health, Education and Welfare to Joseph A. Politch and a Grant-in-Aid from Temple University to Lorraine Roth Herrenkohl. We gratefully acknowledge the technical assistance of Debbie DiMario, Steve Lo Russo, Hady Zal and Cheryl Zink for the conduct of the research.

REFERENCES

1. Allen, T. O. and B. N. Haggett. Group housing of pregnant mice reduces copulatory receptivity of female progeny. *Physiol Behav* **19:** 61–68, 1977.
2. Beckhardt, S. and I. L. Ward. Reproductive functioning in the prenatally stressed female rat. *Dev Psychobiol*, in press.
3. Chapman, R. H. and J. M. Stern. Maternal stress and pituitary-adrenal manipulations during pregnancy in rats: effects on morphology and sexual behavior of male offspring. *J Comp Physiol Psychol* **92:** 1074–1083, 1978.
4. Dahlöf, L., E. Härd and K. Larsson. Influence of maternal stress on offspring sexual behavior. *Anim Behav* **25:** 958–963, 1977.
5. Dunlap, J., J. E. Zadina and G. Gougis. Prenatal stress interacts with prepuberal social isolation to reduce male copulatory behavior. *Physiol Behav* **21:** 873–875, 1978.
6. Herrenkohl, L. R. Prenatal stress reduces fertility and fecundity in female offspring. *Science* **206:** 1097–1099, 1979.
7. Herrenkohl, L. R. and J. A. Politch. Effects of prenatal stress on the estrous cycle of female offspring as adults. *Experientia* **34:** 1240–1241, 1978.
8. Herrenkohl, L. R. and J. B. Whitney. Effects of prepartal stress on postnatal nursing behavior, litter development and adult sexual behavior. *Physiol Behav* **17:** 1019–1021, 1976.
9. McGill, T. E. Sexual behavior in three inbred strains of mice. *Behavior* **19:** 342–350, 1962.
10. McGill, T. E. Studies of the sexual behavior of male laboratory mice: effects of genotype, recovery of sex drive, and theory. In: *Sex and Behavor*, edited by F. A. Beach. New York: John Wiley, 1965.
11. Paris, A. and J. A. Ramaley. Effects of short-term stress upon fertility. I. Before puberty. *Fertil Steril* **24:** 540–545, 1973.
12. Politch, J. A. and L. R. Herrenkohl. Prenatal stress reduces maternal aggression by mice offspring. *Physiol Behav* **23:** 415–418, 1979.
13. Rhees, R. W. and D. E. Fleming. Effects of malnutrition, maternal stress, or ACTH injections during pregnancy on sexual behavior of male offspring. *Physiol Behav* **27:** 879–882, 1981.
14. Sach, B. D. and D. R. Lumia. Comment: is stress due to shipment of animals a confounding variable in developmental research? *Dev Psychobiol* **14:** 169, 1981.
15. Turner, B. B. and A. N. Taylor. Effects of postnatal corticosterone treatment on reproductive development in the rat. *J Repro Fertil* **51:** 309–314, 1977.
16. Ward, I. L. Prenatal stress feminizes and demasculinizes the behavior of males. *Science* **143:** 212–218, 1972.
17. Ward, I. L. Sexual behavior differentation: prenatal hormonal and environmental control. In: *Sex Differences in Behavior*, edited by R. C. Friedman, R. M. Richart and R. L. Vande Wiele. New York, John Wiley, 1974.
18. Ward, I. L. Exogenous androgen activates female behavior in non-copulating, prenatally stressed male rats. *J Comp Physiol Psychol* **91:** 465–471, 1977.
19. Whitney, J. B. and L. R. Herrenkohl. Effects of anterior hypothalamic lesions on the feminized sexual behavior of prenatally-stressed male rats. *Physiol Behav* **19:** 167–169, 1977.