Effects of prenatal stress on the estrous cycle of female offspring as adults

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Summary. Stress during gestation significantly increased estrous cycle length in female offspring as adults, primarily by lengthening the estrus-metestrus stage. Therefore, prenatal stress may disturb the hormonal milieu of the female fetus during a critical hypothalamic differentiation stage.

Prenatal stress deleteriously affects reproductive functions in male offspring as adults. Specifically, stress during gestation feminizes and demasculinizes the sexual behavior of male rats in adulthood[1]. In addition, prenatal stress appears to alter the neuroanatomical or biochemical organization of the male brain toward that of the female brain in the anterior hypothalamus[2], medial preoptic nucleus and median eminence[3,4]. Electrolytic lesions of the anterior hypothalamus, which significantly reduce feminine sexual behavior in female but not male rats, were found to reduce significantly the number of feminine sexual responses performed by prenatally-stressed males[2]. Moreover, as revealed by sensitive microdissection procedures in combination with microassays for brain catecholamines, prenatal stress was found to reduce significantly steady state concentrations of the neurotransmitter norepinephrine in the medial preoptic nucleus and median eminence of late pregnant rats and in male offspring as adults[3,4]. These brain regions regulate neuroendocrine control mechanisms for gonadotropin release from the anterior pituitary gland[5,6]; they also play a role in sexual behavior[7] and are target sites for gonadal steroids[8,9]. With regard to endocrine function, it was reported that prenatal stress suppresses the characteristic elevation of serum prolactin in response to ether stress in males in adulthood[10]. These alterations in endocrine function in the adult may result from prenatal stress effects on the in utero development of neural mechanisms that regulate the hormonal response to ether stress.

Although a considerable amount of work has been reported concerning the effects of prenatal stress on the reproductive functions of males, very little research has been directed toward the effects of prenatal stress on females[11]. Nevertheless, when exposed in utero to the same prenatal stressors as male counterparts, prenatally-stressed female offspring as adults exhibit significant changes in brain biochemistry[4]. Concentrations of the neurotransmitter dopamine are extraordinarily high in the arcuate nucleus[4]. The tuberoinfundibular dopaminergic system, of which the arcuate nucleus is a part, is involved in a neuronal-hormonal negative feedback system which regulates gonadotropin release from the anterior pituitary gland[6]. The present experiment was conducted therefore to determine whether prenatal stress might disrupt at least one reproductive function in female rats in adulthood, namely their estrous cycles.

Materials and method. 20 primiparous, pregnant Sprague-Dawley rats, weighing about 250 g were obtained from Zivic Miller (Allison Park, Pa.) 1 week before stressing. They were housed singly under a standard 12 h light/dark cycle and maintained on food and water ad libitum. On day 14 of gestation, 10 pregnant females were selected at random and subjected to 3 45-min stress sessions per day through day 21. Each 45-min stress session was followed by a rest period of equal duration in the home cage. The stressors were simultaneous bright light, heat and restraint administered according to methods modified from Ward[1]. Specifically, females to be stressed were placed individually in 18×8 cm semicircular Plexiglas restraining cages grouped in rows under 4 bright incandescent lights which produced a surface illumination of more than 400 ft candles and surface temperature of 34 °C. The remaining 10 pregnant females were left unhandled in their home cages. On the day of birth, litters were cross-fostered according to a 2×2 experimental design to control for postpartal rearing experience[12]. At 21 days of age, prenatally-stressed and nonstressed offspring were weaned, segregated by sex and housed 2 per cage. At approximately 60 days of age, all animals were placed in single cages. Beginning at 102 days of age, 28 offspring ($n = 7$ in each of the 4 prenatal/rearing conditions) were selected at random and observed for 25 days for stage of the estrous cycle by the method of vaginal lavage conducted at 10.00 h daily.

Results. The estrous cycles of the prenatally stressed females were significantly longer than those of the nonstressed control animals (table). Further analysis of the individual stages of the estrous cycle showed that prenatal stress increased estrous cycle length mainly by significantly increasing the length of the estrus-metestrus stage (table).

Discussion. The findings of the present study agree with those of Paris and Ramaley[13], who found that heat stress

Effects of prenatal stress on the length of the estrous cycle (days ± SEM)

Measures	Prenatally stressed females reared by Females prepartally Stressed (n = 7)		Nonstressed (n = 7)	Prenatally nonstressed females reared by Females prepartally Stressed (n = 7)		Nonstressed (n = 7)
Stages						
Proestrus	1.29 ± 0.18		1.71 ± 0.31	1.07 ± 0.07		1.34 ± 0.17
Combined groups		1.5 ± 0.18			1.21 ± 0.10	
Estrus-metestrus	6.26 ± 1.35		9.11 ± 3.73	2.57 ± 0.43		2.88 ± 1.22
Combined groups		7.68 ± 1.95*			2.73 ± 0.62*	
Diestrus	3.14 ± 0.59		2.00 ± 1.01	4.40 ± 1.14		2.19 ± 0.50
Combined groups		2.57 ± 0.58			3.30 ± 0.67	
Total cycle length						
Mean cycle length ± SE	10.69 ± 0.78		12.68 ± 3.06	7.31 ± 0.92		6.41 ± 0.99
Combined groups		11.68 ± 1.54**			6.86 ± 0.66**	

* $p < 0.05$ (analyzed by 2-way analysis of variance). Significant stress rearing interactions occurred at each stage ($p < 0.05$). Rearing alone was significant during diestrus ($p < 0.05$). ** $p < 0.005$ (analyzed by 2-way analysis of variance).

introduced postnatally significantly increased the length of the estrus-metestrus phase. That irregularities in gonadotropic hormone release were involved in the effects of postnatal heat stress in the former report[13] were supported by the additional findings that postnatal heat stress delayed vaginal opening, increased the length of time to conception and depressed the onset of pregnancy. Measurements of gonadotropin secretion were not made in the present study, nor were they reported for the prepubertally heat-stressed mice[13]. However, Benson and Morris[14] exposed adult rats to 4-h periods of heat stress daily during days 7–11 of pregnancy and reported that the adrenals were hyperactive, as indicated by elevated weights and elevated serum corticosterone; moreover, pituitary and serum follicle-stimulating hormone, which normally begins to fall by day 12 of pregnancy, remained high.

Recently Ward and Weisz[15] reported that prenatal stress significantly alters circulating levels of gonadal and adrenal steroids during prenatal and early postnatal periods. Furthermore, prenatal stress significantly increases steady state concentrations of dopamine in the arcuate nucleus of female offspring as adults by 153%[4]. Concentrations of catecholamines in the arcuate nucleus are known to influence gonadotropin secretion from the anterior pituitary gland[6]. It is possible therefore that prenatal stress disturbs the hormonal milieu of the female fetus during a critical hypothalamic differentiation stage, resulting in a disruption of estrous cycling in adulthood.

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PRO EXPERIMENTIS

An improved method of transplantation on chicken chorioallantoic membrane (CAM)

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Summary. An improved method for grafting on the chicken CAM is described and compared with other CAM grafting methods.

The first use of the chorioallantoic membrane (CAM) of the chick embryo for transplantation studies probably dates back to the experimental work of Murphy and Rous[2]. Since then, improvements of the CAM grafting technique have been introduced by various investigators[3–5]. Embryo containing eggs, incubated for 8–10 days, are in the best condition for receiving grafts to the chorioallantois[4,6]. By transplantation on CAM, usually much larger and more complex structures can be grown than is possible by isolating organs in vitro[6]. However, the development of the transplants on CAM does not always occur as regularly as with the latter method[7]. Our experiments were undertaken with the aim to improve the CAM transplantation technique, classically described by Harris[8].

Material and methods. The CAMs of fertile eggs from White Leghorn chickens, incubated for 8–10 days at 38.5 °C, were used in this study. On these CAMs, whole embryonic chicken or quail ovaries, testes, adrenals or parts of these organs were transplanted for 8–10 days. A 1st group of transplants were grafted according to Harris[8], a 2nd group with O'Hare's modified technique[7], and a 3rd group with our method.

Results and discussion. The method of Harris[8], when applied as such, often gives very irregular results. Indeed, in a variable number of cases, the host embryo dies during the days following the transplantation. The reason for this death is not always clear. Some sets of chicken eggs seem to be very sensitive, other not at all. Death of the host embryo can usually be avoided by: 1. puncture of the egg shell over the air space, near its highest point (the major blood vessel branches of the chorioallantois, after their determination by candling, being oriented to a topmost position); 2. the creation of the artificial air space above the chorioallantois[5], whilst the blunt pole of the egg is slightly elevated. The oblique position of the egg on its holder may not be altered before the puncture hole over the air space is sealed with transparent tape. By this procedure, the contents of the egg are prevented from bulging excessively into the air space. Another drawback of the classical CAM grafting technique is, that graft 'takes' are erratic, with many grafts undetectable at the end of the transplantation period[7]. O'Hare seems to have overcome this difficulty by placing the grafts between a small piece of cellulose ester (Millipore) filter and the surface of the CAM. This method ensures a more direct contact of the transplant with the chorionic ectodermal layer of the CAM. This facilitates the penetration of the graft by the host's blood vessels. Indeed, after the transplantation, as the CAM ages, its capillary network migrates through the chorionic ectodermal layer of cells[9]. In principle the method of O'Hare is excellent; however it has 2 drawbacks: 1. after the application of a piece of cellulose ester filter material on top of the transplant, the latter is no longer visible and its further evolution can no longer be followed at the surface of the CAM; 2. the