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Prenatal Stress May Alter Sexual Differentiation in Male and Female Offspring

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Introduction

The sexual differentiation model of the brain states that the hormonal milieu, not the genome, determines sexual dimorphism in an inherently female brain [2]. Essentially, the principle states that normal patterns of sexual behavior and gonadotropin secretion are established by adulthood as a function of the presence or absence of androgens during critical perinatal sexual differentiation stages. Two lines of evidence support this view. Firstly, chemical or surgical castration of genetic males during perinatal life feminizes and demasculinizes reproductive morphology, physiology and behavior. Secondly, exposure of genetic females to androgens during a critical developmental stage masculinizes and defeminizes reproductive processes. The severity of the masculinizing and defeminizing action of perinatal androgens (more particularly, the potent metabolite estradiol) depends on the amount and timing of the hormone. The purpose of the following paper is to present the case that maternal stress may influence sexual differentiation in female offspring, and that androgens may mediate prenatal stress effects.

Maternal Stress and Central Nervous System Changes in Male Offspring

The possibility that environmental factors may influence sexual differentiation in the male rat was suggested by *Ward's* [13] observations

that repeated whole-body restraint of pregnant dams under bright lights during the last trimester of gestation demasculinized and feminized the sexual behavior of males. The prenatal stress syndrome in males, characterized by diminished copulatory patterns and increased lordotic behavior potentials, was believed to develop from diminished exposure of the fetal male to the gonadal androgen, testosterone.

The first evidence that prenatal stress may alter morphological development in the central nervous system (CNS) of offspring was provided by *Whitney and Herrenkohl* [16]. The then current belief on the neural basis of sexual behavior was that masculine sexual behavior appeared to be mediated by a system involving the preoptic nucleus and medial forebrain bundle, and that feminine sexual behavior involved the anterior hypothalamus, habenula and medial central hypothalamus. On this basis, *Whitney and Herrenkohl* [16] asked whether 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. They reported that anterior hypothalamic lesions that reduced lordotic behavior in female rats similarly reduced lordotic performance in prenatally stressed rats but had no effect on sexual performance of sham-lesioned prenatally stressed males and other control males.

Maternal Stress and Brain Catecholamines

The findings of *Whitney and Herrenkohl* [16] led to more detailed experiments by *Moyer* and coworkers on changes in concentrations of catecholamines (CA) in discrete brain regions and nuclei of pregnant rats under stress and in their offspring as adults [11, 12]. The effects of maternal stress on CA concentrations in 24 discrete areas associated with norepinephrine (NE)- or dopamine (DA)-containing pathways in the brains of rat offspring as adults were examined by combining microdissection procedures with sensitive radioenzymatic assays for NE and DA. Pregnant rats were exposed to stressors of heat, restraint and bright light during the third trimester of pregnancy whereas control mothers remained unhandled in the home cage. Pregnant rats were restrained in 18×8 cm semicircular Plexiglas cages under 4 bright incandescent lights that produced a surface illumination of 400 ft-cd and a surface tempera-

ture of 34 °C (the standard restraint-heat stress procedure in all our experiments). Stress sessions occurred from day 14 to day 21 of gestation 3 times daily for 45 min starting at 10 a.m., with alternating 45-min periods of rest in the home cage.

Unlike the effects of hormones on sexual differentiation, prenatal stress does not appear to produce marked long-lasting alterations in gross reproductive morphology. Birth weights in general are lower in stressed than in nonstressed offspring [8]. By adulthood, however, there are no apparent gross anatomical or morphological differences between stressed and control offspring. Nevertheless, concentrations of brain CA markedly differ.

The first evidence of interest was that maternal stress altered CA concentrations in male offspring as adults. Prenatal stress reduced NE in the medial preoptic nucleus and median eminence of male offspring by 38 and 49%, respectively. Because these CA changes in male offspring [12] paralleled CA changes in pregnant mothers under stress [11], and coincided with locations of steroid-sensitive receptors in the brain, in regions that regulate both sexual behavior and gonadotropin release, we postulated that stress-induced CA changes in male offspring as adults may be associated with the feminization and demasculinization of sexual behavior.

Secondly, maternal stress altered CA concentrations in female offspring as adults. Prenatal stress increased NE in the entorhinal cortex by 50%, reduced DA in the periventricular nucleus by 34%, and increased DA in the arcuate nucleus by 153%! This extraordinary increase in the steady-state concentration of DA in the arcuate nucleus was most startling and encouraged the examination of prenatal stress effects on reproductive functions in female offspring. For the reason that DA content of the arcuate nucleus regulates prolactin release from the adenohypophysis, adverse reactions of prenatal stress on pregnancy and lactation were expected.

The final evidence supported sex differences in concentrations of CA. Normal adult males had higher concentrations of NE in the paraventricular nucleus (94%), median eminence (64%), dorsal bundle (52%), and entorhinal cortex (46%) and lower concentrations of DA in the caudate nucleus (27%) and periventricular nucleus (53%) than did normal adult females. Sex differences in CA concentrations may be related to differences between normal males and females in steroidal hormones.

Maternal Stress and Reproductive Dysfunction in Female Offspring

When pregnant rats are exposed to periodic stress, female offspring do not show normal reproductive processes in adulthood [5–7]. (*Ward's* [14] negative findings with respect to prenatal stress effects on reproduction in female offspring may be due to the fact she used only 200 ft-cd of light stress whereas we use 400 ft-cd.) Only a small percentage of female offspring of mothers exposed to restraint and bright lights during gestation produce viable young. *Herrenkohl* [5] reports that prenatal stress produces a syndrome in female offspring characterized by diminished reproductive capabilities in adulthood (estrous cycle disorders, spontaneous abortions and vaginal hemorrhaging during pregnancy, stillbirths and neonatal mortality, low birth weight young).

To rule out the possibility that prepartal stress-induced disturbances in the behavior or lactational performance of the mother during the postnatal period were the primary causes of the reproductive deficits in the offspring, cross-fostering procedures were used between and within treatment groups [5, 8]. Prenatally stressed offspring still continued to differ from nonstressed offspring independent of rearing condition. Nevertheless, prepartally stressed mothers did not differ significantly from nonstressed mothers in latency of litter retrievals or duration of nursing behavior regardless of whether they were rearing prenatally stressed or nonstressed offspring. Thus prenatal stress appeared to affect later reproduction in offspring not by disrupting postnatal rearing conditions but by altering the fetus, probably by changing the hormonal milieu.

The findings of the effects of prenatal stress on the estrous cycle of female offspring suggested a pattern strongly reminiscent of androgen effects on ovarian activity. In the hundreds of prenatally stressed female offspring we have examined in our laboratory to date, a majority exhibit estrous cycle irregularities [7]. In these females, prenatal stress nearly doubles estrous cycle length, mainly by increasing the length of the estrous-metestrus stage. A good proportion of the females remaining in the same age range show insignificant variations in estrous cycle regularity, and a rare percentage exhibit polyfollicular ovaries. These few females are sterile and in persistent estrus.

Prenatal stress effects on estrous cycles remind one of the observations of *Harlan and Gorski* [3, 4] on anovulatory syndromes. The full anovulatory syndrome, with sterility and persistent estrus, characteris-

tically results from exposure of genetic females to high doses of testosterone proprionate at the peak of the critical sexual differentiation stage. On the other hand, females exhibiting the delayed anovulatory syndrome, which results from exposure to relatively low doses of androgen later in the critical period, show reproductive cycles for some time following puberty. Subsequently, they lose cyclic function, become anovulatory and show persistent vaginal estrus. As young adults, these animals have been described as changing their 'neuroendocrine sex' with respect to gonadotropin secretion [4]. The percentage of lightly androgenized females with polyfollicular ovaries increases with age, from 12% at 45 days to 91% at 120 days [3]. It is our current belief that the prenatal stress syndrome in females, characterized by diminished reproductive capabilities in adulthood, may result from increased exposure to androgens in utero.

Maternal Stress and Prenatal Androgens

The prenatal stress syndrome in males is characterized by a demasculinization and feminization of sexual behavior in the absence of effects on reproductive morphology [13]. *Ward and Weisz* [15] postulated that the prenatal stress syndrome in males may result from a desynchronization between CNS maturation and patterns of testosterone secretion by the testes during fetal life. In particular, they observed that a surge in plasma testosterone characteristic of normal fetal males on day 18 or 19 fails to occur at the same stage of gestation in stressed males, but occurs prematurely (on day 17).

The most parsimonious explanation at the current time with respect to female offspring is that the prenatal stress syndrome in females may result from increased exposure of fetal females to androgenic steroids. The source of androgen is not known but could include (a) the fetal adrenals: restraint-heat stress markedly elevates maternal body temperature and thereby may affect the fetus directly [1]; (b) the maternal adrenals: restraint-heat stress produces maternal pathology and maternal adrenocortical response [1]; or (c) the fetal testes: this intriguing possibility is suggested by the observation that masculinization of fetal females can be mediated by proximity to fetal males [17]. In the latter circumstance, exposure to androgen could occur locally, at the level of adjacent amniotic membranes. Alternatively, since uterine blood flow in the rat is in the

direction of the cervix toward the ovary, masculinizing hormones secreted by *stressed* fetal males may be carried via the uterine vasculature to female littermates located further downstream [10].

That prenatal androgen exposure may be the mechanism by which maternal stress alters the development of the CNS in offspring is supported by preliminary evidence from experiments currently underway in our laboratory on the effects of prenatal stress on the morphological development of the sexually dimorphic component (SDN) of the medial preoptic nucleus. Evidence abounds that the morphological connectivity of preoptic-anterior hypothalamic neurons is altered by neonatal androgen exposure [2]. Recently *Gorski* [2] has shown that volumetric measurements of the SDN of the male rat brain are 5–8 times larger than those of the female. Gonadectomy of either male or female after day 21 does not influence the volume difference, but castration of the neonatal male results in a significantly smaller nucleus. Conversely, androgenization of the neonatal female by exogenous hormones results in a significantly larger nucleus as compared with that of the normal adult female. Using restraint-heat stress during gestation and examining the volume of the SDN in male and female rats at 30 days of age, we have preliminary evidence that the volume of the SDN in stressed males is smaller than in normal males, and that the direction of differences is reversed in females [2]. On the basis of a small sample to date, volumetric measurements according to the procedures of *Jacobson* et al. [9] appear to be larger in stressed than in nonstressed females. Should these findings remain reliable, evidence for volumetric shifts in the size of the SDN in stressed offspring could reflect differences in exposure to prenatal androgens.

Conclusions

Prenatal stress produces a syndrome in male offspring characterized by diminished copulatory performance and increased lordotic potential. In female offspring, the syndrome appears to involve diminished reproductive capabilities in adulthood (estrous cycle disorders, spontaneous abortions and vaginal hemorrhaging during pregnancy, stillbirths and neonatal mortality, low birth weight young). Evidence is presented that prenatal stress alters (a) the functional organization of the medial preoptic nucleus in males toward that of the female direction, and (b) neurochemical concentrations in critical brain regions associated with

gonadotropin release and sexual behavior in male and female offspring. The most parsimonious explanation of these findings is that maternal stress influences sexual differentiation of the brain by altering exposure of fetal males and females to endogenous androgenic steroids.

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