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Prenatal Stress Feminizes and Demasculinizes the Behavior of Males

Abstract. *Male rats were exposed to prenatal or postnatal stress, or both. The prenatally stressed males showed low levels of male copulatory behavior and high rates of female lordotic responding. Postnatal stress had no effect. The modifications are attributed to stress-mediated alterations in the ratio of adrenal to gonadal androgens during critical stages of sexual differentiation. Specifically, it appears that stress causes an increase in the weak adrenal androgen, androstenedione, from the maternal or fetal adrenal cortices, or from both, and a concurrent decrease in the potent gonadal androgen, testosterone.*

The critical role of androgen during perinatal development on the differentiation of adult sexual behavior potentials has been clearly demonstrated. Male rats deprived of androgen prenatally in injection of the antiandrogenic drug, cyproterone acetate, or neonatally by castrating on the day of birth, display less male copulatory behavior and more female lordotic patterns than normal males (*1*). Conversely, female rats exposed to exogenous androgen during critical perinatal developmental stages show male-like copulatory and ejaculatory patterns while female receptivity is partially or totally impaired (*2*).

In the normal male, the differentiating androgen is presumably testosterone secreted by the fetal and neonatal testes. There is, however, an alternate source of androgen, namely, the adrenal cortex. Among other androgenic steroids, the adrenals secrete small quantities of testosterone and large amounts of the less potent androstenedione (*3*). The possible functional significance of this apparently redundant androgen source has not been investigated. However, since the adrenal cortex under certain pathological conditions releases sufficient androgen to virilize human females (*4*), its role in the differentiation of healthy individuals may have been underestimated. This possibility is worth consideration in view of the large increases in adrenal 17-ketosteroid output during severe stress. The amount and ratio of androgens measured in plasma and urine of stressed animals differ from those of normal subjects. Exposure to a variety of stressors—including shock avoidance, living in overpopulated colonies, and adrenocorticotropin (ACTH)—decreases both testicular size and plasma and urine testosterone concentrations but increases the amount of androgen secreted by the adrenal cortex (*5*). Presumably the increased adrenal androgen is androstenedione since 80 to 85 percent of adrenal androgen is in this form in the normal organism (*3*). The fetal adrenal cortices also are highly secretory and respond to stress and ACTH (*6*), and the placenta is permeable to corticosteroids and ACTH (*7*); thus the fetus is exposed to maternal hormones as well as its own.

Sexual differentiation in male rats stressed during critical prenatal and postnatal developmental stages appears to take place in the presence of large amounts of the weak adrenal androgen, androstenedione, rather than under the primary influence of testosterone. The resulting behavioral potentials would be expected to resemble those obtained by other experimental manipulations which decrease functional testosterone titers. Male behavior should be reduced and female behavior enhanced. In a study designed to test these proposals, 14 time-mated Sprague-Dawley rats were stressed daily during three 45-minute sessions during days 14 to 21 of gestation by being restrained in 7 by 3 inch (18 by 8 cm) semicircular Plexiglas tubes across which 200 foot-candles (2150 lumens per square meter) of light were directed. This treatment produced piloerection and substantial amounts of urination and defecation. Nine control mothers were housed in an adjacent vivarium and were not handled. Half of the prenatally stressed litters and four of the control litters were then given daily postnatal stress from days 1 to 10 of age; stress consisted of three 30-minute sessions during which each male pup of a given litter was placed in a separate compartment of a plastic ice cube tray mounted on a vibrating metal rack. At about 90 days of age, all males were given 30-minute weekly tests with estrous lure females for spontaneous behavior. Each animal continued to be tested until he had either ejaculated or had failed to copulate for 6 weeks. The number of incomplete and complete intromission patterns as well as of ejaculations was recorded.

The results are presented in Table 1. A marked reduction was obtained in the percentage of prenatally stressed animals capable of showing the ejaculatory response. The postnatally stressed group did not differ from the control group, nor did the combination of pre-

natal and postnatal stress enhance the effect above that produced by prenatal stress alone. For the most part, the prenatally stressed groups simply did not attempt to copulate. Those animals initiating mating usually did so by the second or third test. Only one male in each group copulated without emitting an ejaculatory response. If the pattern appeared it was normal. Standard measures such as the mean number of copulatory responses emitted preceding the first ejaculation, the time from the first copulation to the ejaculatory response, and the duration of the postejaculatory interval were not significantly different among responding animals of the different groups.

The males were then castrated and, after a 10-day recovery period, given 5 weekly injections of 0.1 mg of estradiol benzoate followed 42 hours later by 1 mg of progesterone. Beginning on the second week, female receptivity tests were given before the progesterone injection and 2, 4, 6, and 8 hours after. The number of times each animal was mounted by a vigorous male during a 10-minute period and the number of lordotic responses were recorded. Behavior was rated on a 7-point qualitative scale used in this laboratory with 1 indicating minimal and 7 maximal receptivity (*8*). Female behavior ratings recorded during the four tests are summarized in Table 2. All groups were mounted about an equal number of times, but the two prenatally stressed groups averaged over 3 times as many lordotic responses as the control group (Mann-Whitney U, $P < .01$). Postnatal stress had no significant effect on this or any of the other measures of female behavior. The enhanced tendency of the prenatally stressed groups to show female behavior is also reflected in the larger mean percentage of tests on which at least two lordotic responses were emitted and by the superior quality measures. Postnatal stress combined with prenatal stress did not increase the effect above that induced by prenatal stress alone.

Close inspection of the quality measure indicates that, although the level of female behavior shown by the prenatally stressed males was higher than that of normal males, it was lower than that of receptive females. No soliciting behavior, darting, or ear wiggling, typical of strous female rats, were seen; and the males only occasionally held the lordotic posture after the vigorous stud male had dismounted. On the other hand, like estrous females, the males were tense, did not resist being mounted and displayed high-quality lordotic responses which were held as long as the stud remained mounted. Control and postnatally stressed males generally resisted being mounted, but when they permitted it they showed only slight curvature of the neck and back.

Table 1. Percentage of males which ejaculated or copulated on at least one of six tests with an estrous female. Binomial tests of results against the expected probability of control group gave the following results: prenatal stress, $P < .001$; pre- and postnatal stress, $P < .03$.

Stress treatment	*N*	Percentage	
		Ejaculated	Copulated
Control	11	64	73
Postnatal	12	58	66
Prenatal	19	21	26
Pre- and postnatal	10	30	40

With the exception of the group stressed in both ways, responsivity in all groups increased from a low on the first test to a stable level during the last three weekly tests. There was no increase in responding as a function of number of hours after progesterone injection in any of the groups.

The present data support the hypothesis that exposure of pregnant rats to environmental stressors modifies the normal process of sexual behavior differentiation in male fetuses by decreasing functional testosterone and elevating androstenedione levels during perinatal development. During stress conditions plasma testosterone emanating from the gonads decreases while adrenal androstenedione rises (*5*). The molecular structure of the two androgens being very similar, it is postulated that the two hormones compete for the same receptor sites. Since androstenedione is a less potent androgen than testosterone, the decreased male copulatory ability and increased lordotic potential seen in the prenatally stressed animals of the present study would be expected. The relative difference in potency between testosterone and androstenedione has been repeatedly demonstrated. When injected into intact males, both hormones cause decreased testicular size; they also maintain prostate and seminal vesicle weights in hypophysectomized males. However, testosterone is superior to androstenedione in producing these effects (*9*). Further, the feminization of behavior of male rats castrated on the day of birth is not blocked by simultaneous injections of androstenedione as it is by administration of testosterone propionate (*10*).

It is postulated that sexual differentiation occurs under the influence of a dual system. Normally, in males, gonadal steroids would assure the establishment of male copulatory potentials and suppress female lordotic behavior. However, under a variety of unfavorable environmental conditions which have in common the ability to activate the adrenal cortical response to stressors, the normal amount and ratio of testicular to adrenal androgen is altered, and, along with it, the relative potency of androgenic stimuli under which the tissues mediating sexual behavior differentiate. The resulting alterations in sexual behavior provide the basis for an effective population control mechanism, since offspring so affected would not possess the behavioral repertoire necessary to contribute to population growth. Thus, the environment, by triggering or failing to trigger an adrenal stress response, may control the reproductive capacity of successive generations of differentiating fetuses and, thereby, population size. Since male behavior was tested in intact animals, it is not clear whether the modifications in behavior are attributable to stress-induced deficiencies in adult endogenous androgen levels, peripheral sex structures, central nervous system potentials, or some combination of these. Further experiments are in progress to try to delineate the locus of the modification. The female behavior differences, however, were observed in castrated subjects given constant hormone replacement and, therefore, probably reflect central nervous system modifications.

Although sexual differentiation in the

Table 2. Summary of lordotic behavior.

Stress treatment	*N*	Mean lordotic responses	Mean times mounted	Tests* receptive (%)	Median† highest quality
Control	7	2.7	22.1	36	1
Postnatal	8	4.0	21.1	53	1
Prenatal	14	8.8	25.8	73	2
Pre- and postnatal	8	8.4	22.6	88	2

*Tests on which a minimum of two lordotic responses were emitted. †Median of the highest quality lordotic pattern emitted on the four test days.

rat spans both the prenatal and postnatal periods of development, in the present study, stress was effective only during prenatal differentiation. This is not too surprising since it is known that newborn rats pass through a stress-nonresponsive period for the first 10 days after birth (*11*). Paradoxically, while the newborn rat lacks the normal adrenal response to environmental stress, its adrenals show an increased sensitivity to ACTH (*11*). We are currently testing the possibility that by stressing the mother, enough ACTH or adrenal steroids can be transferred to the pup through the milk to influence the process of sexual differentiation which, in the rat, continues to about 10 days of age.

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Olfactory Bulb Units: Activity Correlated with Inhalation Cycles and Odor Quality

Abstract. *Single olfactory bulb units were studied in two macrosmatic species of rodents under conditions intended to preserve the cyclical stimulation which normally accompanies nasal breathing. Patterns of unit activity related to the inhalation cycle were observed in all animals, often in the absence of specific stimuli, and could not be explained in simple mechanical terms. Distinctive changes in these patterns occurred in response to certain odors, and were generally independent of changes in the overall firing frequency. These findings indicate that a change in the overall firing frequency of unit discharges is neither a necessary nor sufficient measure of responsiveness to odors in the rodent olfactory bulb, and that stimulus-specific temporal distributions of unit firing may be involved in olfacto-endocrine activities.*

Among mammals from mouse to monkey, olfacto-endocrine factors figure prominently in the control of critical activity patterns upon which individual and species survival depend. In many species of rodents, carnivores, and primates, scented glandular secretions (pheromones), produced under the combined influence of interoceptive and exteroceptive stimuli, influence in turn the endocrine cycles and the reproductive, territorial, or social behavior of conspecific animals (*1*). Pheromones appear to exert their effects in at least two ways. First, they are distinctive cues or signals and, as such, trigger specific responses from other members of the species. Anyone who has tried to take an estrous bitch for a walk around the block will testify to the potent attractant effects of certain hormone-dependent scents. Second, pheromones normally present within the immediate environment may exert long-term effects. That is, they contribute to the stable background necessary to maintain essential consummatory activities. It is a notorious fact that the absence of familiar odors, or the presence of unfamiliar ones, may interfere with normal patterns of such varied activities as eating, sleeping, copulation, and maternal behavior. Furthermore, specific mammalian pheromones, produced under endocrine control, have been implicated in the regulation and maintenance of normal reproductive cycles (*2*).

In most mammals, pheromones and other airborne stimuli are continuously swept in wave-like fashion across the primary olfactory receptors deep within the nasal cavity. Under what we call resting conditions, when the normal respiratory rhythm provides a basis for repetitive and periodic sampling of the olfactory background, the normal sampling epoch coincides with the nasal inhalation phase of each respiratory cycle. Whenever a distinctive change in the quality of the inhaled air is detected during regular nasal breathing, most mammals react by sniffing, a sign of active olfactory exploration which tends to take precedence over any routine consummatory activities. Sniffing involves increase in the mean frequency of nasal inhalations and in the overall rate of odor sampling. These changes are accompanied by corresponding decreases in the duration of each sampling epoch. Thus, under both resting and active conditions, olfactory stimulation remains an essentially periodic process. This periodic patterning of nasal air flow may itself play a role in the processing of olfactory information.

In an attempt to elucidate the mechanisms whereby animal scents exert their effects upon behavioral and endocrine activities in rodents, we have been studying the responses of single olfactory bulb units to various odors in two macrosmatic species: the Syrian golden hamster (*Mesocricetus auratus*) and the prairie deermouse (*Peromyscus maniculatus bairdii*). For reasons already noted, precautions have been taken to preserve the periodicity of odor stimulation which occurs under natural conditions. We report two findings consistently obtained in each of more than 100 animals of both species and sexes, tested under various conditions and at ages ranging from two weeks to six months. These are (i) a general tendency for olfactory bull units to exhibit a definite temporal pattern of firing with respect to particular phases of the nasal inhalation cycle and (ii) the occurrence of stimulus-specific changes in this pattern in the presence of various odors. Such changes often occurred without a corresponding alteration in the overall frequency of unit firing. We conclude that stimulus-specific differences in the