Maternal Stress Alters Plasma Testosterone in Fetal Males

Abstract. *Titers of testosterone in plasma were determined by radioimmunoassay in male rat fetuses of stressed and control mothers on days 17, 18, 19, 21, and 23 (the day of birth) after conception. In fetuses of stressed mothers, testosterone concentrations were highest on day 17, declined on days 18 and 19, and then remained unchanged. In the control fetuses, testosterone increased from relatively low concentrations on day 17 to the highest amounts on days 18 and 19, and then declined. Thus, the persistence of feminine and impaired masculine sexual behavior in male offspring of stressed mothers could be due to the absence of a surge of circulating testosterone during days 18 and 19 after conception, a period postulated to be critical in the development of the central nervous system in the rat.*

A basic requirement for the expression of normal male sexual behavior in adult rodents is that central nervous system (CNS) tissues mediating this function undergo masculinization and defeminization during critical perinatal stages of development (*1*). Male offspring of rats subjected to stress from days 14 to 21 of pregnancy show a persistence of female behavioral potentials and an inability to exhibit normal male copulatory patterns in adulthood (*2, 3*). Thus the processes involved in masculinization and defeminization appear to have been compromised in the male fetuses of stressed mothers.

Since sexual differentiation of reproductive behavior in males depends on the secretion of adequate amounts of testosterone by the fetal testes (*1*), we postulated that the behavioral syndrome characteristic of males born of stressed mothers is due to an attenuation of testosterone during the perinatal period critical for sexual differentiation of the CNS (*2, 4*). This possibility was suggested by numerous observations that stress decreases concentrations of circulating testosterone in adult males of a variety of species, including the rat (*5*). This report shows that stress also alters testosterone concentrations in male rat fetuses. In particular, a surge in plasma testosterone characteristic of normal male fetuses (*6*) fails to occur at the same stage of gestation in male fetuses of stressed mothers.

Sprague-Dawley female rats (Sprague-Dawley, Madison, Wisconsin) were time-mated between 1500 and 1600 hours and randomly assigned to a stressed or a control group. Beginning on day 14 of gestation (day of mating is day 0), females in the stressed group were placed, three times daily for 45 minutes each time, in 13 by 6 by 8 cm Plexiglas animal holders illuminated by two 150-W floodlights (2150 lm/m^2). This treatment was given at 0900, 1300, and 1700 hours during the dark phase of the animals' light-dark cycle (lights were off from 0800 to 2000 hours); mothers and their fetuses subjected to this treatment show significant transient elevations in plasma corticosterone concentrations. Control females were not handled.

Mothers from the control and the stressed groups were killed on days 17, 18, 19, and 21 of gestation between 1500 and 1630 hours. The pregnant females were decapitated and their uteri excised and placed on ice. Trunk blood collected in heparinized capillary tubes from individual fetuses was centrifuged, and the plasma from animals matched by sex, age, and treatment was pooled to a volume of 0.30 ml per sample (*6*). Blood from several litters was pooled to make up a single sample, but no litter contributed blood toward more than one sample.

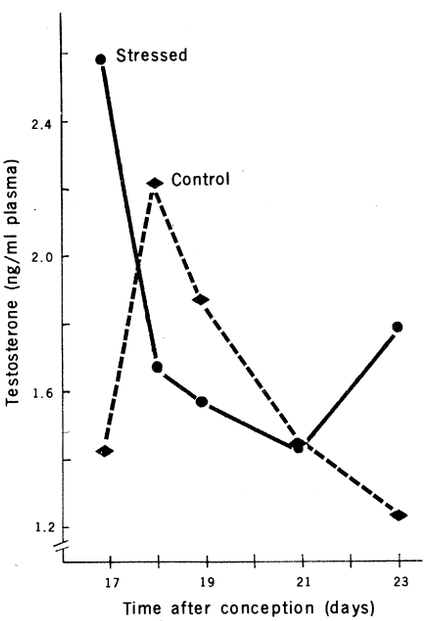

Fig. 1. Mean concentration of testosterone in plasma samples obtained from male rat fetuses between day 17 of gestation and day 23, the day of birth. The following number of samples were assayed in the control and stressed groups in each age category. Day 17: control, N = 12, standard error of the mean (S.E.M.) = 207; stressed, N = 11, S.E.M. = 582. Day 18: control, N = 13, S.E.M. = 211; stressed, N = 10, S.E.M. = 157. Day 19: control, N = 18, S.E.M. = 204; stressed, N = 20, S.E.M. = 129. Day 21: control, N = 22, S.E.M. = 201; stressed, N = 14, S.E.M. = 156. Day 23; control, N = 11, S.E.M. = 170; stressed, N = 12, S.E.M. = 300. Stressed and control groups were significantly different ($P < .05$) from one another on days 17 and 18 of gestation.

One group of litters was killed on the day of birth, day 23 after conception. Approximately 1 to 8 hours after delivery, litters from control mothers and from mothers that had been subjected to stress daily from days 14 through 21 of gestation were removed individually from the nest and decapitated; their blood was collected as it was from the fetuses. To minimize stress before blood collection, pups remained with their mothers up to the time of decapitation. The mothers were anesthetized (Equithesin, intraperitoneally) to prevent them from becoming agitated when a pup was removed and, thus, stressing the remaining pups. Testosterone was measured in the plasma samples by radioimmunoassay, as described previously (*6, 7*).

Mean titers of testosterone in plasma of male fetuses from stressed mothers deviated significantly from titers of control fetuses on 2 of the 5 days when samples were taken (Fig. 1). A two-way analysis of variance yielded a significant interaction between treatment by days ($P < .01$). Male fetuses of stressed mothers had significantly higher testosterone concentrations than control males on day 17 of gestation ($t = 1.94$, d.f. = 22, $P < .05$). On day 18 male fetuses of stressed mothers had significantly lower testosterone concentrations than control males ($t = 2.00$, d.f. = 22, $P < .05$).

Concentrations of testosterone in plasma changed significantly over days in both the stressed ($P < .05$) and the control ($P < .05$) groups, but the pattern of change between the two groups differed. In control animals, testosterone titers rose between days 17 and 18 of gestation ($P < .05$) and then declined from day 18 to day 21 ($P < .05$). The concentrations on days 21 and 23 did not differ significantly from each other. In males of stressed mothers testosterone titers declined significantly from day 17 to day 18 ($P < .05$) and thereafter remained relatively stable. The values obtained on days 18, 19, 21, and 23 were not significantly different from one another.

Our data demonstrate for the first time, to our knowledge, that environmental stressors acting on the pregnant mother can alter the plasma testosterone titers of male fetuses, thereby modifying sequential hormonal events determining normal masculine differentiation of sexual behavior. In a previous study (*6*), differences in testosterone concentrations between normal male and female fetuses were found to be maximal on days 18 and

0036-8075/80/0118-0328$00.50/0

19 of gestation and to decrease thereafter. Day 18 was also the only time from days 17 to 23 after conception when the testosterone concentration in individual pools from normal males was consistently higher than in matched female littermates. On day 17, mean testosterone concentrations in plasma of control males were not yet higher than in control females. The most striking change in males from stressed mothers is that they do not show the surge in testosterone on day 18 of gestation that characterizes unstressed males. Rather, stressed males have their highest testosterone titers on day 17.

On the basis of the above observations, we propose that day 18 of gestation represents a distinct and critical point in the process of sexual differentiation of the fetal rat brain. Specifically, we suggest that adequate masculinization of behavioral potentials requires, and may be initiated by, exposure of the developing CNS to an acute surge of testosterone secreted by the testes at a critical stage of fetal ontogeny, which is day 18 after conception in the rat. Behavioral masculinization is completed by sustained exposure through day 5 postpartum of the now androgen-sensitized CNS to concentrations of testosterone not markedly higher than those of normal females. The abnormal pattern of sexual behavior in male offspring of stressed mothers could result from the lack of testosterone surge on fetal day 18. After day 19, males from stressed mothers have testosterone titers comparable to those of unstresssed animals; however, their CNS may not be sufficiently sensitive to respond to the lower concentrations of testosterone secreted at these later stages. The finding that testosterone titers are significantly higher in male fetuses of stressed mothers than in control males on day 17 of gestation suggests that the testosterone surge is not eliminated in stressed fetuses, but occurs prematurely. Thus, the prenatal stress syndrome, characterized by impaired adult male copulatory behavior and an enhanced female lordotic potential, could result from a desynchronization between CNS maturation and patterns of testosterone secretion by the testes during fetal life.

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7. Details of the radioimmunoassay procedure can be found in (*6*). Briefly, after addition of tritiated testosterone (2600 to 3600 dis/min), 200-μl samples of plasma were extracted with 1.5 ml benzene saturated with deionized water and then with 1.5 ml methylene dichloride or twice with cyclohexane. The pooled extracts, containing two drops of olive oil, were dried under nitrogen and dissolved in 1 ml of isooctane. The extracts were chromatographed on Celite columns. The dried testosterone fractions were reconstituted in 1 to 2 ml of methanol and incubated overnight with [1,2,6,7-^{3}H]testosterone (approximately 4000 count/min) and an antiserum to testosterone (conjugated at the C-3 position). Free and bound steroids were separated from each other by activated charcoal. The samples were centrifuged at 7000 to 8000*g* for 10 minutes. The radioactivity in the supernatant was counted in a solution of toluene, liquiflor, and 2 percent methanol in a Packard scintillation counter. Duplicate measures on 25-, 50-, and 100-μl portions of the plasma samples were made. Representative samples from the different age and treatment groups were introduced into each assay.
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Interchangeability of Stress and Amphetamine in Sensitization

Abstract. *In view of similarities between the behavioral, biochemical, and electrophysiological effects of amphetamine and stress, we tested the hypothesis that presentation of a stressor, mild tail pressure, can sensitize an animal to the later effects of amphetamine, and vice versa. Our findings supported this hypothesis and suggest that amphetamine and at least some stressors may be interchangeable in their ability to induce sensitization. The data raise the possibility that stress might be a common variable contributing to both amphetamine psychosis and some forms of schizophrenia.*

In humans, repeated consumption of large doses of amphetamine or other stimulants often results in the progressive development of a psychotic syndrome notable for its resemblance to paranoid schizophrenia (*1*). Animals similarly treated with constant doses of stimulants also show a progressive enhancement (that is, sensitization) of certain behaviors [for example, stereotypy and locomotion (*2*)]. This apparent similarity between the sensitizing effects in animals and the gradual evolution of paranoid symptoms in humans has led to the suggestion that repeated stimulant administration may provide insight into some of the factors underlying amphetamine-induced psychosis and perhaps schizophrenia itself (*2, 3*). One such factor could be "stress." Acute psychotic episodes can be precipitated by stress in some schizophrenic individuals (*4*), and stress has been shown to reinstate amphetamine psychosis in abstinent individuals during remission (*5*). Moreover, the similarity in the neurochemical effects of stress and stimulants has prompted the suggestion that stimulants may produce their psychotogenic actions by imitating the effects of stress on the organism (*6*). These considerations, coupled with our own observations of a marked similarity between the acute behavioral, pharmacological, biochemical, and electrophysiological responses to a particular stressor, mild tail pressure (TP), and amphetamine administration (*7, 8*), led us to ask whether stress and amphetamine are interchangeable with regard to sensitization. We now report that repeated TP stress is sufficient to produce a virtually identical sensitization of amphetamine-induced sniffing as that seen during long-term amphetamine administration. Conversely, a single injection of amphetamine can result in a persistent sensitization of TP-induced behavior.

Male Sprague-Dawley rats (Zivic-Miller, Pittsburgh) weighing 200 to 300 g were used in these experiments. Mild TP (approximately 80 to 110 pounds per square inch) was applied 2.5 cm from the tip of the tail to 18 animals by means of a

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