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Biological predictors of masculine sexual behavior in prenatally stressed and nonstressed rats

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Sexual activity values, volume measures of the sexually dimorphic nucleus of the preoptic area (SDN-POA), and testosterone levels of a sample of 30 male offspring selected from litters born to control and prenatally stressed mothers were intercorrelated. The resulting correlations were then submitted to partial correlational analyses in order to determine the relative contributions of anatomical structure and testosterone levels in mediating sexual behavior. It was observed that SDN-POA volume was a better predictor of sexual activity than were testosterone levels.

The sexual dimorphic nucleus of the medial preoptic area (SDN-POA) of the rat brain is an intensely staining region within the preoptic hypothalamus. Gorski and collaborators have shown that this region is several times larger in normal male rats than in normal females (Gorski, Gordon, Shryne, & Southam, 1978; Gorski, Harlan, Jacobson, Shryne, & Southam, 1980) and that it can be used reliably to identify male and female brains. Recently, we reported that prenatal treatments of heat/light/restraint stress reduced the volume of the SDN-POA in male rats. In addition, prenatally stressed males generally showed reduced levels of sexual activity and lower plasma testosterone levels in comparison with those of nonstressed control males (Anderson, Fleming, Rhees, & Kinghorn, 1986). Although the correlations between SDN-POA volume, testosterone levels, and sexual activity showed an interesting pattern of relationships among these variables, because each variable is positively related to the other, the correlations could have been spuriously high. Accordingly, in the present report, a sample of male offspring from control and prenatally stressed mothers was drawn, and SDN-POA volume measures were intercorrelated with testosterone levels and indices of sexual activity in these animals. The obtained correlations were then submitted to a partial correlational analysis (McNemar, 1955) to examine the relationship between two variables while partialing out the variance contributed by the third variable. The relative strength of the volume of the SDN-POA and plasma testosterone levels as predictors of masculine sexual behavior in male rats was determined in this manner.

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METHOD

Subjects

The sample of animals used in this study comprised 30 Sprague-Dawley male rats, 130-140 days of age. Half of the sample (n = 15) were selected from the litters of 8 nonstressed mothers and the remaining half (n = 15) were selected from the litters of 6 prenatally stressed mothers. Mothers of both control and stressed animals were housed in Plexiglas maternity cages with room temperature at 24° C, provided with water and Purina Rat Chow ad lib, and maintained on a reversed 12:12-h light:dark cycle (lights were off from 0800 h to 2000 h).

Procedure

Mothers of prenatally stressed males were subjected to treatments of heat/light/restraint stress during the third trimester of gestation (Day 14 of pregnancy to parturition, plug day = 0). This stress consisted of restraint three times daily for 45-min periods in 13×6×8 cm semicircular transparent Plexiglas chambers which were placed under the illumination of two 150-w floodlamps (2,150 lm/m²). The mothers of control animals remained in the maternity cages through pregnancy, parturition, and weaning of the offspring. At Day 25 postpartum, control and stressed offspring were sexed and weaned; males were separated and housed singly.

Beginning at 75 days of age, both prenatally stressed and control male offspring were given 30-min tests once a week for spontaneous sexual behavior. Ovariectomized females made receptive by hormonal injections (0.1 mg of estradiol benzoate followed 42 h later by 1.0 mg of progesterone) were used as behavioral lures. Testing was continued for 7 weekly sessions, or until a male ejaculated. Sexual activity was defined as at least one ejaculation during the 7-week test period.

One week following the completion of the mating tests, prenatally stressed and control males were anesthetized with a 2:1 mixture of ketamine-acepromazine (0.1 mg/kg), and approximately 2 ml of blood were drawn from each animal via cardiac puncture. The plasma was separated and stored at −20° C until analyses of testosterone concentrations were carried out by radioimmunoassay with kits purchased from Wien Laboratories, Inc.

Immediately after drawing blood, the animals were perfused through the left ventricle under gravity with saline for 4 min and then with 10% formalin for an additional 4 min. Their brains were then removed from the cranium and stored in 10% formalin. After fixation for at least 4 days, the brains were embedded in paraffin, sectioned serially in the DeGroot plane at 50 μm and stained with thionin. Following these histological procedures, three investigators, using a protocol similar to that described by Gorski et al. (1978), independently traced the boundaries

of the SDN-POA on the left side of every slide in which it appeared. The volume of the SDN-POA was obtained by summing the area of the individual nuclear tracing for each animal, taking into account section thickness and the magnification of the microprojector used to make the tracings.

RESULTS

Correlations between SDN-POA volume, sexual activity, and testosterone levels were computed by using Pearsonian, point biserial, and partial correlation procedures. The data from both groups of animals were combined into single distributions of scores for each of the three variables under investigation for purposes of the correlational analyses.

The point biserial correlation coefficient between the volume of the SDN-POA and sexual activity for the 30 animals sampled was $r_{pb} = .91$, $p < .001$. That is, a strong relationship existed between the size of the SDN-POA and the likelihood of observed masculine sexual behavior. In addition, correlation coefficients determined between plasma testosterone and sexual activity ($r_{pb} = .68$, $p < .001$) and between SDN-POA volume and testosterone levels ($r = .76$, $p < .001$) indicated a strong relationship between the levels of circulating testosterone and the likelihood of observed sexual activity and a strong positive relationship between the size of the SDN-POA and the level of testosterone.

Partial correlation coefficients were calculated, using these data to determine the relative strength of SDN-POA volume and plasma testosterone level as predictors of masculine sexual behavior. The partial correlation coefficient between SDN-POA volume and plasma testosterone level with the effects of sexual activity held constant was $r = .47$, $p < .02$. The partial correlation coefficient between plasma testosterone level and sexual activity with the effects of SDN-POA volume held constant was $r = -.04$, $p > .05$. The partial correlation between SDN-POA volume and sexual activity with the effects of plasma testosterone level held constant was $r = .82$, $p < .001$.

DISCUSSION

It is clear from the partial correlation data that the best predictor of sexual activity is the volume of the SDN-POA, and that the volume of the SDN-POA is significantly related to testosterone level. Of particular interest is the lack of correlation between sexual activity and testosterone levels when SDN-POA volume was held constant by partial correlational procedures. It is counterintuitive to think that testosterone levels and sexual behavior are not related. Our own point biserial correlations support such reasoning ($r_{pb} = .68$) and are consistent with published data (Leshner, 1978). However, in a three-way correlation, when a source of variance is statistically removed from the other sources of variance, the resulting correlation portrays the fundamental relationship between the remaining two members of the triad. Although it is clear that testosterone must be present for normal sexual behavior to occur (Leshner, 1978), it has been determined that an optimal level of testosterone is required for sexual activity and that levels appreciably greater than a threshold level do not have a correspondingly greater impact on sexual behavior (Beach & Fowler, 1959; Harding & Feder, 1976). Thus testosterone levels do not appear to be as robust a predictor of sexual behavior as does SDN-POA volume. The role of the SDN-POA in the expression of sexual behavior is yet to be determined, but the present results strongly implicate this nucleus as a relevant structure.

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