and HGPRT activities in the two clones that were PGK-negative excluded the accidental loss of the entire active X chromosome. A partial deletion of the X chromosome, involving only the PGK locus, might be postulated because of the peripheral location of this locus on the long arm of the X chromosome. The finding of a normal karyotype for both PGK-negative clones was at least evidence against the occurrence of a large deletion. In order to account for complete deficiency of enzyme activity in cell lysates from different subcultures of both clones, a deletion should already have been present at the very beginning of the cloning procedure, that is, in two of the individual cells that gave rise to the 22 clones examined. Spontaneous chromosome breaks have never been reported in freshly established diploid cultures of human fibroblasts.

The most obvious interpretation of the data, therefore, is that only one of the two PGK alleles is expressed in each cell. Consequently, the structural gene for human PGK is involved in X chromosome inactivation, as is true with all of the other X-linked loci that have been investigated by cloning [G6PD (*8*), Hunter's locus (*13*), HGPRT (*14*), and $\alpha$-galactosidase (*15*)]. Contrary to what takes place in marsupials, preferential inactivation of the paternal PGK locus does not appear to occur in man. A conclusion cannot be drawn at this stage, however, as to whether the excess of clones bearing an active paternal X chromosome reflects a situation already existing in vivo or is the result of selection in vitro. This question can best be examined by cloning cells from a heterozygote for two alleles with the same biological fitness, such as the carriers of the electrophoretic variants described by Chen *et al.* (*2*). Unfortunately, these subjects were not available for the present study. The present data and the variable expression of PGK deficiency in other tissues of our heterozygous donor are consistent with the current hypothesis of random X chromosome inactivation at an early stage of embryonic development as the usual mechanism for dose compensation of X-linked genes in man (*16*, *17*).

The demonstration that the PGK locus undergoes inactivation is of particular interest in view of its known chromosomal location. This investigation, to our knowledge, provides the first evidence that the long arm of the normal human X chromosome is involved in X inactivation. Moreover, it seems likely that two of the other loci involved in X inactivation (G6PD and HGPRT) are located on the short arm of the human X chromosome (*4*) at an appreciable distance from one another, as suggested by the high incidence of meiotic recombination between them (*18*) and by their mitotic separation in somatic cell hybrids (*19*). Thus, the conclusion emerges that the entire human X chromosome may, indeed, be involved in the mechanism of X inactivation. This hypothesis (*16*) and its numerous variations (*20*) will be more easily evaluated when a good cytological map of the human X chromosome becomes available.

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## Sexual Receptivity: Facilitation by Medial Preoptic Lesions in Female Rats

Abstract. *Lesions in the medial preoptic area of ovariectomized female rats reduced the quantity of estrogen needed to induce sexual receptivity in these animals. In addition, the number of days over which receptive behavior could be elicited after a single initial estrogen injection and with subsequent daily progesterone treatment was significantly increased by lesions in the medial preoptic area. These findings support the view that estrogen acts to reduce an inhibitory action that is tonically exerted by the medial preoptic area on pathways mediating estrous behavior.*

In most mammals female sexual behavior is characterized by a cyclic responsiveness to sexual approaches by the male that vary between receptivity and rejection. For some time it has been known that these changes in female sexual activity are produced by the interaction of varying amounts of steroid hormones and the nervous system. Hormonal, neural, and behavioral factors that influence sexual receptivity have been studied most extensively in the rat. In this species the medial preoptic–anterior hypothalamic region is considered a critical integrative center for mediating sexual receptivity (*1*). Long-term implants of estradiol seem most effective in reinstating behavioral estrus when placed in this area (*2*) and preferential uptake of radioactively labeled estradiol

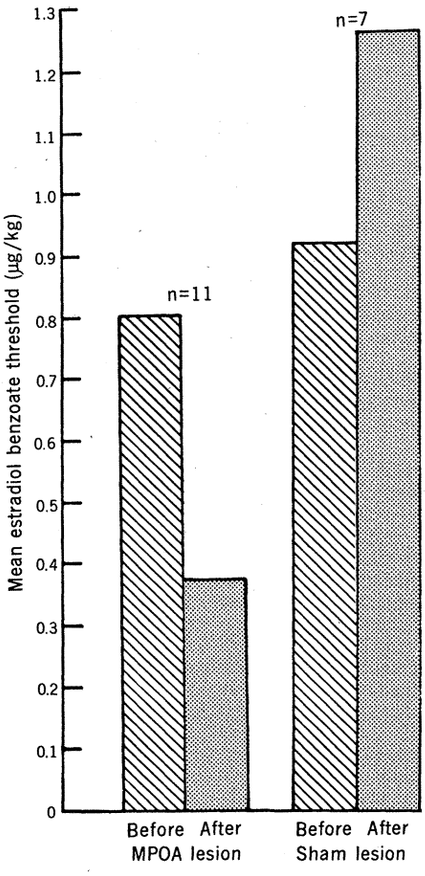


Fig. 1. Mean estradiol benzoate thresholds before and after MPOA lesions or sham operations.

is shown by cells in the medial preoptic area (MPOA) although other areas in the hypothalamus and limbic system also exhibit affinity for this steroid (*3*).

It is generally presumed that the role of the medial preoptic–anterior hypothalamic region in mediating sexual receptivity is reflected in a loss of behavioral responsiveness to ovarian hormones after destruction of this region (*1*). However, most investigations have not treated separately the effects of damage to the MPOA and the anterior hypothalamus. Although lesions to the anterior hypothalamus may eliminate or reduce the capacity for estrous behavior (*4*, *5*), this effect is not unequivocal (*6*). No marked changes in receptivity have been found after lesions restricted to the MPOA (*5*). This conflicts with the finding that estrogen uptake is high in the MPOA and that estrous behavior can be induced by estrogen-loaded cannulas inserted into this region. However, if it is assumed that the MPOA controlled sexual responsiveness by inhibiting lower centers in the final common pathways for estrous behavior, then estrogen might act on cells in the MPOA to reduce this inhibition. We report that after lesions restricted to the MPOA, estrous behavior can be induced by quantities of estrogen which are much less than the amounts required prior to the operation.

Sprague-Dawley female rats (250 g) were ovariectomized and housed with free access to food and water under a 12-hour-on, 12-hour-off light cycle (lights off at noon). Behavioral sensitivity to estrogen was assessed by measuring the intensity of receptivity after estrogen and progesterone injections given at weekly intervals with the quantity of estrogen systematically reduced over successive weeks until lordosis could no longer be elicited. Varying amounts of estradiol benzoate and a constant quantity of progesterone (0.5 mg) were injected 48 and 6 hours, respectively, before behavior tests were conducted (*7*). Sexual receptivity was measured by scoring the lordosis responses elicited by vigorous Long-Evans male rats previously adapted to standard mating arenas (*8*). The quality of each response was rated as either 0 (no concave arching of back), 1, 2, or 3 (slight, moderate, and full arching, respectively), which represented gradations from no lordosis to a maximum response. Tests were terminated after response scores to ten adequate mounts by the male had been obtained (*9*). For each behavior test, the mean response score was used as the receptivity index.

All females were initially injected with a priming dose of estradiol benzoate (40 μg/kg) followed by progesterone (0.5 mg) and the receptivity test. One week later, an injection of 8 μg/kg was given and over successive weeks the estradiol benozate quantity injected was reduced by one-half until a "threshold" criterion had been met. A dose of estradiol benzoate was considered threshold when its associated receptivity index was 0.0 (*10*). After threshold was reached, each subject was assigned to a group for either MPOA or sham lesions. Insofar as possible, the two groups were matched for estradiol benzoate threshold and body weight. Bilateral MPOA lesions were made with a platinum electrode (0.5 mm in diameter) insulated with Epoxylite to within 0.75 mm of the tip (*11*). Subjects in the sham-lesion group received identical treatment except for passage of current. One to two weeks after these operative procedures, hormone injections and behavior testing were resumed beginning with a dose of estradiol benzoate four times the threshold dose prior to the operation. Thresholds were determined with procedures identical to those used in the preliminary testing phase.

The mean estradiol benzoate threshold of the 11 females receiving MPOA lesions was less than one-half the value prior to the operation (Fig. 1; $P < .005$, Wilcoxon test). The seven sham-operated females showed nonsignificant threshold changes in the opposite direction. The use of means to depict changes after MPOA lesions produces a very conservative estimate of the increased responsiveness to estrogen. The median estradiol benzoate thresholds before and after the operations were 1.0 and 0.125 μg/kg and 0.5 and 1.0 μg/kg for the female rats that had received MPOA lesions and sham operations, respectively. This difference between the mean and median figures for the MPOA group can be attributed to one animal that exhibited a threshold increase after the operation. Of the remaining ten

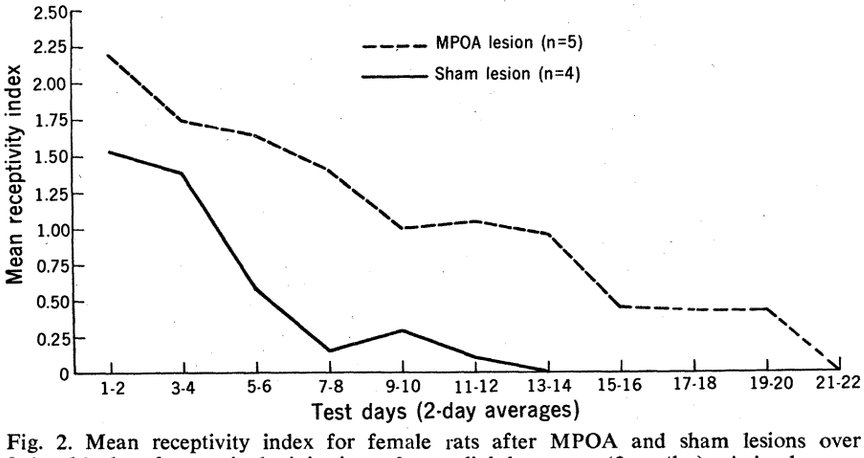


Fig. 2. Mean receptivity index for female rats after MPOA and sham lesions over 2-day blocks after a single injection of estradiol benzoate (2 μg/kg). Animals were given progesterone (0.5 mg) 6 hours before each daily behavior test until sexual receptivity was no longer evident.

animals, eight had thresholds that were 25 percent or less than those prior to operation (four of these dropped to 6.25 percent or lower). Seven of these eight females had thresholds which were 0.125 μg/kg or below (*12*).

All lesions involved at least partial destruction of the MPOA with minimum damage extending into the anterior hypothalamus (*13*). There is no sharp boundary between the MPOA and the anterior hypothalamus, but we have adopted the criterion, used by others, that the boundary is the coronal level at which the anterior margin of the nucleus suprachiasmaticus first appears. In one brain the center of the destroyed field was approximately 0.50 mm anterior (at the level of the diagonal band of Broca) to the lesions of the other animals in the operated group; this female was the only subject showing an increase in estradiol benzoate threshold after the operation.

The significant increase in estrogen sensitivity after destruction of the MPOA suggested that other aspects of behavioral responsiveness to ovarian hormones may have been altered. A second group of ovariectomized females (MPOA lesion, $n = 5$; sham lesion, $n = 4$) was tested to determine the number of days receptivity could be maintained after a single injection of estradiol benzoate with daily progesterone treatments. Each subject was primed with estradiol benzoate (40 μg/kg) and progesterone (0.5 mg) in our standard sequence but was not tested for receptivity (*14*). After 1 week, estradiol benzoate (2 μg/kg) was given and beginning 42 hours later daily injections of progesterone (0.5 mg) were administered, but no additional estrogen was given, and receptivity was assessed each day 6 hours later by the standard behavioral testing method. Progesterone injections and tests were terminated when the receptivity index over two successive days was 0.0.

Subjects with lesions were more receptive than the sham controls (Fig. 2; $P \leq .032$ for test days 1 to 2, 5 to 6, 7 to 8, 11 to 12, and 13 to 14, U test), and this receptivity persisted in the absence of additional estrogen for a much longer period. Measurable receptivity was displayed by females with lesions in the MPOA for a mean of 16.8 days. Three of the five subjects were still responding on day 18. In contrast, sham controls failed to respond after a mean of 8.0 days. This difference was highly significant ($P = .008$, U test) (*15*).

To assess the possibility that estradiol benzoate was being differentially absorbed and metabolized in the two groups, we made vaginal smears on days 7 to 10. The smear patterns did not enable us to differentiate between groups or among individuals within groups displaying different levels of receptivity. Thus, we found no evidence that estrogen was available systemically for longer periods in the subjects with lesions. It would appear, therefore, that the differences between the MPOA and sham groups in both the estradiol benzoate threshold and receptivity duration studies are most consistent with a central nervous system "release" effect.

Our findings provide evidence for an inhibitory control over behavioral estrus. The notion that the lordosis response in the rat is normally held under some degree of inhibition by the central nervous system and that ovarian hormones reduce this inhibition is not a novel idea (*4*, *16*). However our results are the first to implicate a particular neural site as an important component of this inhibition. The changes we find in estradiol benzoate threshold after lesions in the MPOA are consistent with the hypothesis that the MPOA tonically inhibits activity in lower centers and that estrogen acts at the MPOA to reduce this inhibition. Destroying a significant number of inhibitory cells reduces the quantity of estrogen needed to disinhibit those MPOA cells remaining functional (*17*).

The present results should be viewed against the background of several reports that MPOA lesions impair male sexual behavior (*5*, *18*). Therefore, the possibility that the sexual orientation (masculine or feminine) may in part be determined by critical changes in the preoptic area should be considered. This view is consistent with a report (*19*) that a significant sexual dimorphism has been found in the mode of axonal termination within the preoptic area.

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8. Plexiglas-fronted, semicircular mating arenas were 76 cm in diameter and 40 cm in both height and width.
9. In some instances testing was stopped after six responses if all these were scored as either 3 or 0. Testing was also discontinued if the stud male ejaculated on or after response 5. If ejaculation occurred before this, testing was resumed in 5 to 10 minutes.
10. If after any particular dose of estradiol benzoate the receptivity index was 0 but the receptivity index on the preceding weeks' test was 1.0 or greater, the estradiol benzoate dose giving a 0.0 response was repeated; if 0.0 was again obtained this quantity of estrogen was considered a valid threshold.
11. Lesion coordinates from bregma were: anterior-posterior 0.0 mm; lateral, ± 0.5 mm; vertical, — 8.0 mm with skull level between lambda and bregma. Anodal current (2 ma) was passed for 15 seconds using a rectal cathode.
12. By comparison, we have given estrogen threshold tests to 84 ovariectomized Long-Evans female rats. The median threshold obtained was 1.0 μg/kg and only one animal was below 0.5 μg/kg. Seventy-six of these subjects have completed a second threshold test and the median value has remained at 1.0 μg/kg although the mean increased by 6 percent. Thus we have reliable evidence on a much larger group of animals that our method for obtaining estradiol benzoate thresholds in no way involves increased sensitivity to estrogen on repeated threshold tests.
13. B. Powers and E. S. Valenstein, in preparation.
14. All animals had been given hormone injections and behavioral tests for other purposes prior to this experiment but none had received estradiol benzoate and progesterone for a minimum of 3 weeks.
15. Our results may seem to conflict with previous reports concerning behavioral responsiveness to ovarian hormones after lesions in the medial preoptic-anterior hypothalamic region. In addition to the usual lack of distinction between the MPOA and the anterior hypothalamus, these investigations have been oriented toward finding a decrease in sexual performance after the lesions and have not been designed to be sensitive to changes in the opposite direction. Commonly, the estrogen doses used in the past have varied between 25 to 500 μg per animal. By comparison, the estrogen threshold quantities (0.0078 to 2.0 μg/kg) we used range between 1/50 to 1/250,000 of the levels employed in earlier studies. The relatively massive doses used in earlier investigations were likely to have masked any differences between groups with lesions and control groups.
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