Hypothalamic Unit Activity in the Cat: Effects of Estrogen and Vaginal Stimulation[1] (35569)

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(Introduced by L. L. Boyarsky)
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Recent studies concerned with the neural control of ovulation and the expression of sexual behavior have focused upon electrophysiological events in the brain and the influences of hormones upon these events. Two different electrical recording methods have provided valuable information with respect to hormonal and genital sensory feedback upon brain activity: electroencephalographic (EEG) and unit recording techniques. Changes in EEG activity during cervical stimulation have been recorded in the hypothalamus of the rat (1, 2), rabbit (3), monkey (4), and cat (5). In all of these cases, EEG changes were demonstrable in the naturally estrous or estrogen primed animal, but not during anestrus. Barraclough and Cross (6) introduced unit recording methods to such types of studies. These workers and others (2, 7, 8) found that in the rat, cervical stimulation influenced the firing pattern of cells in various hypothalamic areas. Estrogen was shown to selectively depress the response of lateral and anterior hypothalamic cells. Similar observations from the rostral hypothalamus were obtained in the estrogen-primed immature monkey by Chhina and Anand (9).

The cat, which is a reflex ovulator, is an ideal species for studying neural changes associated with ovulation since ovulation can be induced in the laboratory by stimulation of the cervix (10). Up to now, investigations on hypothalamic unit activity in reflex ovulators are quite limited. Reports by Kawakami and Saito (11) and Alcaraz *et al.* (12) showed that units in the hypothalamus changed their firing rates upon cervical stimulation, and that the type of change was dependent upon the estrogen status of the animal. Both these studies indicated that the anterior hypothalamus was one area in which unit activity could be affected.

Lesioning and implantation studies in the cat have implicated the anterior hypothalamus in the control of sexual behavior, while similar studies in the posterior hypothalamus have given predominantly negative results (13, 14). In this report, unit activity of single neurons in the anterior and posterior hypothalamic areas was studied in response to cervical stimulation in the adult female cat under the following conditions: anestrus, ovariectomized, and estrogen-treated ovariectomized. Neurophysiological examination of the posterior hypothalamus was considered to be important since recent anatomical findings in our laboratory indicate that the ultrastructure of this area in the cat is altered with changes in estrogen levels (15). It was felt that such a study might help obtain a better understanding of the physiological significance of two distinct areas of the hypothalamus with respect to integrating afferent impulses from the genital tract and changes in estrogen levels necessary for the induction of ovulation and expression of sexual behavior.

Methods. In these studies, 35 mature female cats ranging in weight from 2.0 to 3.5 kg were used. All experiments were carried out under alpha-chloralose anesthesia (65 mg/kg) with the body temperature maintained at 36–38° by the use of an electric heating pad. Tungsten microelectrodes, measuring 0.5 to 1.5 μ at the tips, were used for recording extracellular unit activity from the

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anterior and posterior hypothalamic nuclei. The stereotaxic coordinates used were based on Snider and Niemer's Stereotaxic Atlas of the Cat Brain (16). The anterior hypothalamic coordinates ranged from A = 12.0–13.0, ML = 0.5–2.0, V = 5.0–8.5; the posterior hypothalamic coordinates ranged from A = 7.5–9.0, ML = 0.5–2.5, V = 5.5–9.5. After passing through a high impedance cathode follower, the responses were amplified with a preamplifier and displayed on a dual-beam oscilloscope. The data were simultaneously stored on magnetic tape and later photographed. At the end of each experiment, a DC current of 4–10 mA was passed through the electrode to produce a lesion for verification of the electrode placement.

Stimulation of the vaginal cervix consisted of short repeated movements of a lubricated Teflon probe. For each unit studied, the following experimental sequence was carried out: (i) a 1-min control period just prior to stimulation, (ii) a 1-min period of stimulation of the vaginal cervix, (iii) a 1-min recovery period, (iv) a second minute of stimulation, and (v) a final 1-min recovery period. As a control for stimulation of the vaginal cervix, stimulation by other means such as stroking the thigh or pinching the tail was employed at the end of each experiment.

Three groups of cats were studied: one group consisted of cats which failed to show any behavioral characteristics of estrus (anestrus group); the second group of cats were ovariectomized at least 2 weeks prior to the recording session and the third group were ovariectomized and pretreated with estradiol benzoate (Progynon, Schering) for 3 days prior to the recording sessions. In order to induce behavioral estrus, estrogen was administered subcutaneously at a dose of 0.08 mg/cat for 2 days before and on the morning of the recording day.

The data were analyzed visually from photographic records. If more than 1 unit was present in a record, only the dominant unit was analyzed. The firing rate of three 10-sec samples of each 1-min period was determined and the degree of change (percentage increase or decrease over the control period) was then calculated. The percentage change was calculated for activity in both the first and second stimulation periods. Only those units which showed a reproducible change in firing rate in conjunction with both stimulation periods were counted as responsive. Small changes (15%) were not included in the analysis. In order to show that the changes were, in fact, elicited by the stimulation, firing rates in the control and first recovery period were compared. The firing rate of all the units included in the study returned to control levels during the recovery period.

It is difficult to compare the magnitude of a percentage increase with a percentage decrease since they do not change in a proportionate manner. Therefore, when considering the magnitude of change over control rates, the median was used as a measure of the average rather than the mean since the mean is highly sensitive to extreme variability (17).

Results. Data obtained from these experiments indicate that stimulation of the vaginal

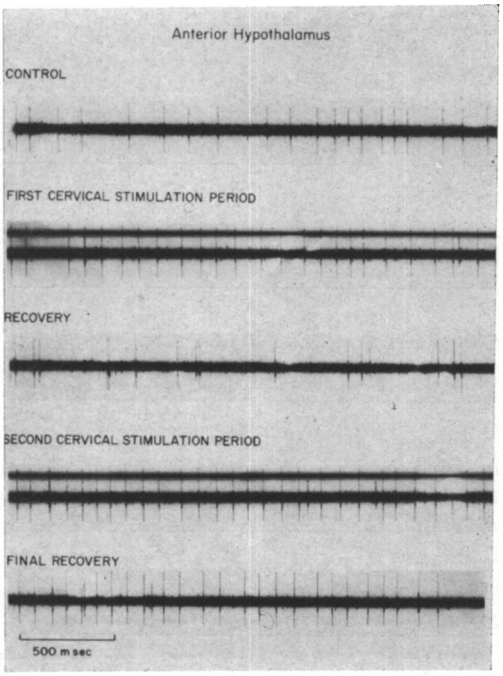

FIG. 1. An anterior hypothalamic cell showing an increase in firing rate during two periods of stimulation of the vaginal cervix.

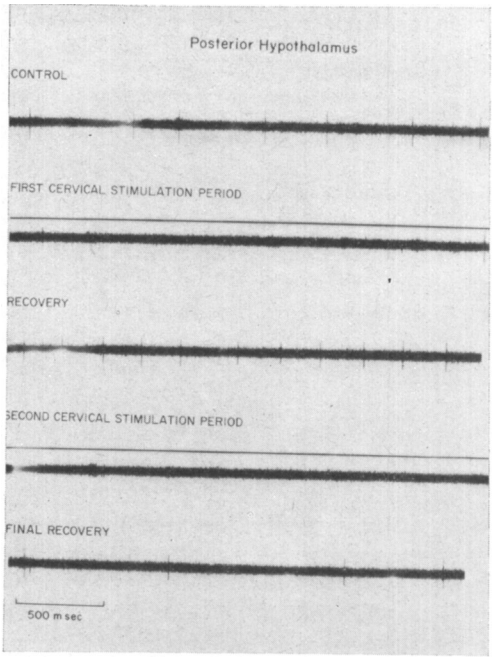

FIG. 2. A posterior hypothalamic cell showing a decrease in firing rate during two periods of stimulation of the vaginal cervix.

cervix can influence the rate of unit activity in both the anterior and posterior hypothalamus. An example of a unit which increased its firing rate is shown in Fig. 1; a unit which decreased is shown in Fig. 2. The units which responded to stimulation of the vaginal cervix were not influenced by the other types of peripheral stimulation employed. The mean firing rates of all groups ranged from 6 to 10 impulses/sec.

Anestrus cats. The changes in hypothalamic unit activity produced by stimulation of the vaginal cervix in the cat are given in Table I. In the anestrus cats, 35% of the cells studied in the anterior hypothalamus showed some change in unit activity; 23% of the cells increased and 13% decreased their firing rate. Records from the posterior hypothalamus showed, in comparison, that only 25% of the cells were affected by stimulation of the vaginal cervix, with 12% showing an increase in firing rate and 12% a decrease.

Ovariectomized cats. When analyzing the responses of the anterior hypothalamic cells during stimulation in the ovariectomized group, it was observed that 47% of the cells studied changed their firing rate, 29% increasing and 18% decreasing. In the posterior hypothalamus, however, the number of cells which were influenced by stimulation was considerably less (26% compared to 47%). Of this total, 13% of the units showed an increase in rate and 13% showed a decrease. It appears that during stimulation of the vaginal cervix there is a greater tendency for increased firing rate in the anterior hypothalamus compared to cells in the posterior area.

Ovariectomized + estrogen-treated cats.

TABLE I. Changes in Hypothalamic Unit Activity Produced by Cervical Stimulation in the Cat.

	No. of cells	No. of cells increasing	% of cells increasing	No. of cells decreasing	% of cells decreasing	% of responsive cells
Anterior hypothalamus						
Anestrus	31	7	23	4	13	35
Ovariectomized	34	10	29	6	18	47
Ovariectomized–estrogen-treated	30	10	33	6	20	53
Total	95	27	28	16	17	45
Posterior hypothalamus						
Anestrus	24	3	12	3	12	25
Ovariectomized	23	3	13	3	13	26
Ovariectomized–estrogen-treated	37	6	16	12	32	49
Total	84	12	14	18	21	35

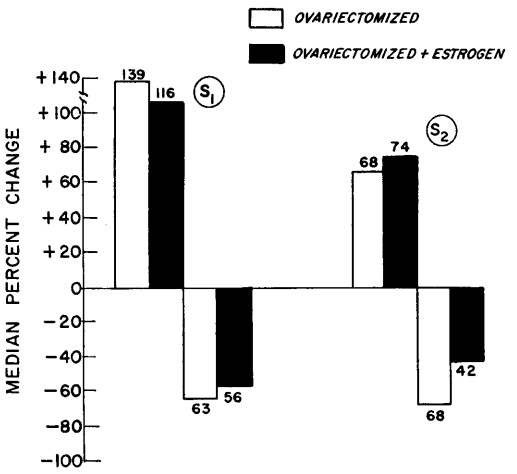

FIG. 3. Histogram of the median percentage change of firing rates of cells in the anterior hypothalamus to stimulation of the vaginal cervix. Responsiveness of cells in ovariectomized cats are compared to estrogen-treated ovariectomized cats: (S_1) first stimulation period; (S_2) second stimulation period. Bars above 0 base represent median percentage change for cells showing an increase in firing rate; bars below 0 line for cells showing a decrease in firing rate.

In the estrogen-pretreated ovariectomized cats (Table I), 53% of the cells in the anterior hypothalamus changed their firing rate during stimulation of the vaginal cervix, 33% increasing and 20% decreasing. In the posterior hypothalamus, the percentage of cells which changed their firing rate was not significantly different from that observed in the anterior (49 versus 53%). However, more cells showed a decrease (32%) in firing rate than an increase (16%). These values are quite different from those found in the posterior hypothalamus in the other two experimental groups. In the anestrus and the nontreated ovariectomized group, the percentage of units showing an increase in firing rate as a result of cervical stimulation was essentially the same as the percentage which decreased.

Ovariectomized versus estrogen-treated ovariectomized cats. When comparing the responses of the ovariectomized and the pretreated ovariectomized groups, there are no appreciable differences seen in the anterior hypothalamus; that is, the percentage of units which increased their firing rate and the percentage which decreased are essentially the same in both groups. However, in the posterior hypothalamus, the pretreatment with estrogen is associated with a greater percentage of units which decrease their firing rate during stimulation of the vaginal cervix.

Figures 3 and 4 represent the median percentage change in firing rate over that in the control period for the ovariectomized and pretreated ovariectomized groups in the anterior and posterior hypothalamus, respectively. Values obtained during both the first and second stimulation periods are shown in Figs. 3 and 4. In the anterior hypothalamus (Fig. 3), during the first stimulation period, the magnitude of increase of firing rate was greater in the ovariectomized group as compared to the estrogen-treated group. However, during the second stimulation period, this difference between the two groups was in the opposite direction. The magnitude of decrease of firing rates was greater in the ovariectomized group than the pretreated during both stimulation periods.

In the posterior hypothalamus, Fig. 4, the

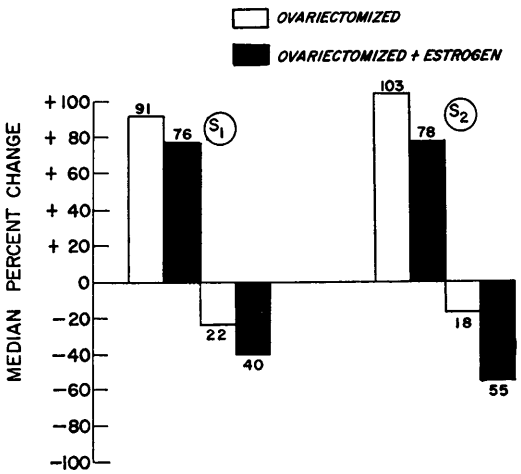

FIG. 4. Histogram of the median percentage change of firing rates of cells in the posterior hypothalamus to stimulation of the vaginal cervix. Responsiveness of cells in ovariectomized cats are compared to estrogen-treated ovariectomized cats: (S_1) first stimulation period; (S_2) second stimulation period. Bars above 0 base represent median percentage change for cells showing an increase in firing rate; bars below 0 line for cells showing a decrease in firing rate.

changes in unit activity observed appeared to follow a definite pattern in both stimulation periods. During the first stimulation period, the magnitude of increase was greater in the ovariectomized group and this trend was repeated during the second stimulation period. The magnitude of decrease was in the same direction during both stimulation periods with much smaller decreases in firing rate noted in the ovariectomized non-estrogen treated cells. Figure 4 shows that in the ovariectomized cats, firing rates increased approximately 100% during the two stimulation periods. In the estrogen-treated group, this increase was approximately 75%. Units showing a decrease did not decrease as much in the ovariectomized group (20%) as compared to the estrogen-treated ovariectomized group where the decrease was approximately 48%. Thus, low levels of estrogen may be associated with a general increase in activity in the posterior hypothalamus.

Discussion. In our experiments, anterior hypothalamic cells in anestrus and ovariectomized cats exhibited both increased and decreased firing rates in response to stimulation of the vaginal cervix with a greater tendency toward increased firing rates. These results are consistent with the findings of Barraclough and Cross (6) for the anterior hypothalamus of the rat. In the cat, however, other workers have recorded anterior hypothalamic units under these conditions and have reported a lack of response (11) or predominantly inhibitory responses (12). Our results in the posterior hypothalamus in anestrus and ovariectomized cats showed that approximately one-half of the responsive units exhibited increased firing rates and one-half exhibited decreased firing rates. Kawakami and Saito (11) and Alcaraz *et al.* (12) did not report on cells from the posterior hypothalamus in their studies on the cat.

The effects of estrogen on hypothalamic activity were studied utilizing ovariectomized cats, since hormonal levels could be best predicted in these animals. Our observations indicate that estrogen treatment does not change the firing pattern of units in the anterior hypothalamus in response to stimulation of the vaginal cervix. These results do not agree with Kawakami and Saito (11) and Alcaraz *et al.* (12) who reported that estrogen facilitated the appearance of excitatory responses to stimulation of the vaginal cervix in the anterior hypothalamus. The differences might be due to the fact that the other workers recorded neural activity from unanesthetized-immobilized cats. In addition, Alcaraz *et al.* (12) utilized the multiunit technique with which overall activity is recorded from an area rather than individual unit responses.

With respect to the posterior hypothalamus, in the present experiments, estrogen-treated ovariectomized cats: (i) increased the number of cells showing a decreased firing rate with stimulation of the vaginal cervix; (ii) increased the magnitude of the response of cells which decreased their firing rate; (iii) reduced the magnitude of the response of cells which increased their firing rate. These findings suggest that increased estrogen levels are associated with decreased hypothalamic activity. Whereas our results were obtained from the posterior hypothalamus, Lincoln and Cross (8) and Chhina and Anand (9) have reported depressed rostral hypothalamic unit activity in the estrus rat and estrogen-primed monkey. EEG studies also suggest that estrogen is associated with a generalized depression of hypothalamic excitability (18).

Since different effects were found in the anterior and posterior hypothalamus, it seems important to consider the implications. The anterior hypothalamus is considered a center for the expression of sexual behavior in the female cat (13, 14). Harris *et al.* (19) showed that implantation of estrogen in the posterior hypothalamus of ovariectomized cats induced sexual behavior. However, studies using more refined estrogen implants suggest that Harris and co-workers' findings were probably due to diffusion (20). As yet, stimulation and lesioning experiments designed to localize hypothalamic centers involved in the release of ovulating hormone have not been carried out in the cat. Our results suggest that the posterior hypothalamus may play a greater role in the regulation of ovulation and sexual behavior than has been postulated. Recent ultrastructural observations in our lab of the posterior hy-

pothalamus of the cat in response to estrogen administration gives support to this view (15). Further investigation as to the significance of the role of this hypothalamic area is necessary.

Summary. Unit activity in the anterior and posterior hypothalamus was studied during stimulation of the vaginal cervix in anestrus, ovariectomized, and estrogen-treated ovariectomized cats. The results were as follows: (i) cells in the anterior and posterior hypothalamus were influenced by stimulation of the vaginal cervix; (ii) there was a greater tendency for increased firing rates in the anterior as compared to the posterior hypothalamus; (iii) estrogen treatment did not change the responsiveness of cells in the anterior hypothalamus; (iv) estrogen treatment was associated with a general depression of unit activity in the posterior hypothalamus. These findings suggest that the posterior hypothalamus may play a role in the regulation of ovulation and expression of sexual behavior in the cat.

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