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BRIEF COMMUNICATION

Hormonal Induction of Behavioral Estrus Modified by Electrical Stimulation of Hypothalamus[1]

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NAPOLI, A., J. B. POWERS AND E. S. VALENSTEIN. *Hormonal induction of behavioral estrus modified by electrical stimulation of hypothalamus.* PHYSIOL. BEHAV. 9 (1) 115–117, 1972.—Sexual receptivity was induced by sequential injections of estrogen and progesterone in ovariectomized female rats which had bilateral, monopolar electrodes permanently implanted into either the medial preoptic area (MPOA) or the medial basal hypothalamus (MBH). Intermittent electrical stimulation of the MPOA during the first 6 hours of estrogen priming significantly reduced the intensity of estrous behavior measured 2 days later. Comparable stimulation of the MBH produced a non-significant increase in receptivity. The use of electrical stimulation to study the ways in which hormones affect central sites of action is discussed.

Electrical stimulation of the brain Estrous behavior Sex Estrogen Medial preoptic area Medial basal hypothalamus

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ALL METHODS for studying the central nervous system loci of hormonally produced behavior changes have some limitations. Systemic injections of hormones offer little or no information about effective brain sites and interpretations based upon combined brain lesions and systemic hormonal administration can be misleading as the changes produced may not be the result of destruction of the site of hormonal action. Autoradiographic studies may provide anatomical information on hormonal uptake, but they offer little help in regard to behavioral regulation. This is particularly true because hormones commonly are taken up by nerve cells in several regions of the brain and the different physiological and behavioral consequences of a particular hormone are often not initiated from the same neural structures. Implantations of hormonally loaded cannulae directly into brain sites necessarily involve complications arising from the possibility of applying non-physiological concentrations and the diffusion of the hormone away from the cannula tip.

In spite of the expressed reservations, all of these methods have contributed useful and complementary information concerning the hormonal regulation of behavior. It is in this context that we describe a new method involving electrical stimulation of the brain to study the central nervous sites of hormonal action. Our technique was developed to study the way in which estrogen and progesterone affect neural tissues to mediate the expression of sexual receptivity in female rats. We reasoned that electrical stimulation of various central sites which selectively accumulate estrogen might produce differential effects on receptive behavior depending on the functional importance of the stimulated area to the regulation of behavioral estrus.

It has been repeatedly demonstrated that in order to produce maximal estrous behavior, estrogen "priming" must precede the triggering action of progesterone when these homones are administered exogenously to ovariectomized females [9] and this same temporal sequence is also characteristic of the patterns of ovarian steroid secretion which occur naturally during the 4-day estrous cycle [8]. Although the site of progesterone action in the CNS is not well understood, considerable evidence suggsts that the medial preoptic area (MPOA) may be critical in mediating the effects of estrogen on sexual receptivity. This is supported by the fact

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that (a) chronic implants of estradiol in the MPOA can reinstate behavioral estrus in ovariectomized females [1, 2, 3], (b) that the MPOA is one of the main sites of estrogen uptake [7], and (c) that lesions in the MPOA dramatically reduce the quantity of estrogen needed to induce receptivity [6]. The findings reported here indicate that electrical stimulation of the MPOA during the period of estrogen "priming" substantially reduces the intensity of sexual behavior measured 2 days later.

MATERIALS AND METHOD

Fifteen female rats of the Long–Evans hooded strain (Simonsen Laboratories, Inc., Gilroy, California) were the subjects for the study. Animals weighing between 250 and 350 g at the beginning of the study were housed in individual cages and maintained under a 12 hr light–12 hr dark illumination schedule.

Bilateral, monopolar electrodes were aimed for the medial preoptic area (MPOA) in 11 animals and for the region of the ventromedial and arcuate nuclei of the hypothalamus, the latter to be referred to here as the medial basal hypothalamus (MBH), in 5 animals. The electrodes were positioned stereotaxically under methoxyflurane anesthesia (Penthrane) using the following coordinates representing, respectively, millimeters behind bregma, to the left and right of the midline, and distance below the top of the skull: MPOA—0.0, 0.5, 8.0; MBH—3.0, 0.5 and 8.9. All coordinates were calculated with the skull level between bregma and lambda. Electrodes were made of 0.25 mm diameter nichrome wire completely insulated except for the cross section at the tip. A bare section of similar wire was inserted 3.0 mm into the brain to serve as an indifferent electrode. Miniature Amphenol connectors were permanently attached to the skull to facilitate rapid hook-up of animals to the stimulating cables.

Animals were ovariectomized (OVX) within 3 days after electrode implantation and on that same day 6 weekly hormonal injection sequences were begun. On weeks 1 and 4, animals were administered a priming sequence consisting of 40 μg/kg 17 β-estradiol benzoate (EB) followed 42 hours later by 0.5 mg progesterone (P). (Progynon Benzoate and Proluton were generously supplied by the Schering Corporation, Bloomfield, New Jersey.) All injections were subcutaneous and hormones were dissolved in sesame oil. The volume of P administered was always 0.1 ml; EB volume in ml was equal to half the body weight in kg. Receptivity tests were conducted during weeks 2, 3, 5 and 6 to determine the behavioral responsiveness to lower doses (4 μg/kg) of EB. Each animal received brain stimulation following the estrogen injection on only one half of its tests. Figure 1 depicts schematically the testing paradigm we used during the weeks on which electrical stimulation was scheduled. Half of the animals received stimulation during weeks 3 and 5 and no stimulation during weeks 2 and 6. The sequence was reversed for the remaining animals. Three animals received only two weekly tests with one following stimulation. One animal received a total of six tests, half of these following stimulation. On appropriate weeks at 0-hr (Fig. 1), all animals were taken from their home cages and were placed in special stimulation chambers with food and water available. Electrodes were attached to flexible cables just prior to the 4 μg/kg EB injection whether or not stimulation was scheduled. If it was scheduled, 10 μA, 60 Hertz sine wave stimulation was begun immediately after the injection with one side being stimulated for 5 sec, followed by a 5 sec no-stimulation period and then 5 sec of current to the opposite electrode. This sequence was repeated for 6 hours. As animals had either bilateral MPOA or MBH electrodes they received electrical stimulation for a total of 3 hours in one of these diencephalic structures, this time being equally divided between the two electrodes. Animals were returned to their home cages at the completion of the 6 hr stimulation period and 36 hr later were given the 0.5 mg P injection followed after 6 hours by the receptivity test.

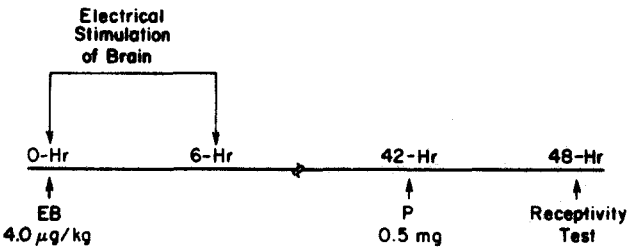

FIG. 1. Schematic diagram illustrating sequence of treatments and tests. Each animal received only one-half of its test with stimulation. EB=17 β-Estradiol Benzoate, P=Progesterone.

Sexual receptivity was measured by scoring the lordosis responses elicited by the mounting activity of vigorous Long–Evans male rats, previously adapted to semi-circular mating arenas (76 cm diameter and 40cm in height and width). A male's behavior had to consist of a discrete mount with palpation of the flanks and rapid pelvic thrusting before the female's response was scored. The degree of lordosis following each acceptable mount was rated on a scale of 0–3. Zero=no concave arching of the back; 1, 2 and 3=slight, moderate and full arching, respectively. Receptivity tests were usually terminated after response scores to 10 mounts by the male had been obtained. In some instances tests were stopped after fewer than 10 mounts. This occurred either when all of the first 6 responses were scored as 0 or 3, or when the male ejaculated on or after the 5th response. If ejaculation occurred before this, testing was resumed in 5–10 minutes. For each weekly behavior test the mean response score (RS) was used as the quantitative index of receptivity.

At the completion of the experiment the animals were sacrificed and following perfusion with formalin and saline the brains were removed. Frozen brain sections were stained with cresylechtviolet and examined microscopically in order to determine the anatomical site of the electrode tips.

RESULTS

The data presented in Fig. 2 are based on receptivity tests obtained from the 11 MPOA animals (22 stimulation and 22 no-stimulation tests) and the 5 MBH animals (8 stimulation and 8 no-stimulation tests). The testing sequence was found not to have influenced the test scores so the data were combined in the appropriate stimulation and no-stimulation categories.

Receptivity scores without stimulation did not differ for the MPOA and MBH animals (RS=1.50 and 1.45, respectively). The MPOA animals receiving stimulation achieved a mean RS of 0.79 representing a 47% reduction from the no-stimulation level ($p<0.05$, 2-tail, Wilcoxon test). In

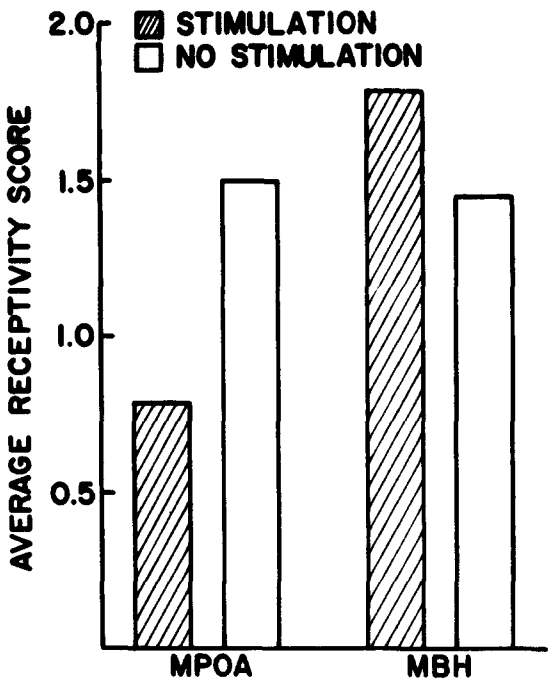

FIG. 2. Receptivity scores of medial preoptic area (MPOA) and medial basal hypothalamus (MBH) groups. Number of stimulation and no-stimulation tests were 22 and 22 for MPOA (N=11); 8 and 8 for MBH (N=5).

contrast, animals with MBH electrodes exhibited a non-significant increase (RS=1.78) in receptivity following stimulation. Therefore, electrical activation of some estrogen receptor sites at a time when they otherwise would be rapidly accumulating the injected estrogen results in a significant reduction in sexual receptivity approximately 48 hours later.

Histological analysis indicated that the MPOA electrodes were all rostral to the MBH electrodes. In general, the MPOA electrodes were found to be located at the border of the MPOA and the anterior hypothalamus. Some of the electrodes were in the anterior hypothalamic area. The MBH electrodes were all more caudal and none were in the anterior hypothalamus. Some of the MBH electrodes were located in the posterior ventromedial region, while other MBH electrodes were located in the posterior hypothalamus.

DISCUSSION

The findings reported here suggest that electrical stimulation of the medial preoptic and anterior hypothalamic region during the time that systemically injected estrogen is taken up by cells in these areas interferes with the priming of estrous behavior in female rats. Comparable stimulation of the more posterior MBH region of the hypothalamus does not have the same consequences. This evidence for anatomical specificity and the fact that the behavior tests were administered 42 hr after the completion of the electrical stimulation both argue against a non-specific action of the stimulation.

Although estrogen is taken up by cells in both the MPOA and the MBH [7], and can induce reasonable levels of receptivity when chronically implanted into either of these areas [2, 3], the present evidence suggests that the effects of estrogen on sexual behavior are mediated by the MPOA. Diffusion of hormone from cannulae in other diencephalic regions to the MPOA could account for the behavioral effects observed with implants outside this area [4].

The technique of stimulating specific brain regions at critical periods may be a useful method for delineating the temporal sequence of physiological events occurring at these sites. In the present context it may be possible to define the time period following availability of circulating estrogen that is necessary for the induction of female sexual behavior. Would it be possible, for example, to interfere with estrogen action on receptivity if the electrical stimulation was not begun until 10 minutes after the injection? Would stimulation of different neural sites have a different time period for maximal effects?

The mechanism by which electrical stimulation of MPOA cells disrupts the later display of receptivity is unknown, but a reasonable assumption is that estrogen uptake processes have been altered. A direct test of this possibility, though, has not yet been made and other alternatives should be considered. It has been suggested that the MPOA, or pathways coursing through it, tonically inhibit lower centers controlling estrous behavior and that estrogen acts to lower this inhibition [6]. Conceivably, electrical stimulation of the MPOA did not interfere with estrogen uptake, but acted to sustain patterns of inhibitory electrical activity.

Electrical stimulation of the MPOA could induce adrenal progesterone secretion. It is known that sufficient quantities of progesterone can antagonize the behavioral effects of estrogen if the progesterone is present during the initial priming actions of estrogen [5]. Although it has not been established that MPOA stimulation produces adrenal progesterone secretion, the possibility that this mechanism accounts for the behavioral effects we observed could be assessed by repeating the experiment with adrenalectomized animals.

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