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Effects of Paced Coital Stimulation on Termination of Estrus and Brain Indoleamine Levels in Female Rats

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ERSKINE, M. S. AND M. J. BAUM. *Effects of paced coital stimulation on termination of estrus and brain indoleamine levels in female rats.* PHARMAC. BIOCHEM. BEHAV. 17(4) 857–861, 1982.—Serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) levels were measured in brain regions of ovariectomized, steroid-primed rats at the end of behavioral estrus. At the onset of behavioral estrus three groups of rats received coital stimulation which included (1) mounts without penile intromission only, (2) temporally-controlled (paced) mounts with intromission, and (3) non-paced mounts with intromission. Within 12 hr after the initial treatment, sexual receptivity was significantly lower in females given paced intromissions than in the other two groups. Brain stem 5-HT and 5-HIAA concentrations were significantly higher in females which received intromissions, regardless of whether or not they were paced than in females given mounts only. Hypothalamic and cortical concentrations of 5-HT and 5-HIAA were equivalent in the two groups. These results suggest that the termination of behavioral estrus is not associated with increased metabolic activity in central serotonergic neurons.

Brain indoleamines Paced coital stimulation Estrous cycle Ovariectomized rats

NUMEROUS reports suggest that activity in serotonergic neurons normally inhibits the expression of feminine sexual behavior (lordosis) in the female rat. Treatment of ovariectomized, estrogen-treated rats with pharmacological agents which enhance serotonergic activity [7, 8, 10, 21, 22, 25, 26] generally decreased lordotic responsiveness, while serotonin (5-HT) synthesis inhibitors [8, 26, 35], receptor blockers [8, 34, 36], or depletors [25] have been shown to facilitate lordotic behavior. It has been suggested that ovarian steroids, in particular progesterone (P), may facilitate estrous behavior by inhibiting serotonergic activity [5, 7, 8, 14, 18, 25]. However, neurochemical data demonstrating effects of ovarian steroids on serotonergic neurons are not wholly consistent with the postulated inhibitory influences of 5-HT on lordotic behavior. Chronic depletion of whole brain 5-HT by maintenance on a tryptophan deficient diet did not affect the ability of estradiol (E) or E in combination with P to induce sexual receptivity in ovariectomized rats [24], and 5-HT levels or turnover in large brain areas or in individual hypothalamic nuclei have not been shown to differ dramatically in ovariectomized rats given behaviorally effective dosages of E or E and P [4, 8, 25, 26, 28, 33]. Increases in serotonin receptor$_1$ concentrations also occur at a time following E administration when lordotic behavior would be elevated [1].

On the hypothesis that increased activity in serotonergic neurons might inhibit estrous behavior during particular times when levels of sexual receptivity are in the process of declining, the present experiment involved measurement of regional 5-HT and 5-HIAA levels toward the end of the period of sexual receptivity when lordosis is becoming less frequent and intense. In several species including the rat, cervical-vaginal stimulation received during mating increases the rate at which lordotic responsiveness wanes at the end of estrus [2, 3, 13, 15, 19]. We therefore initially attempted to modify the rate of estrus decline by varying the amount of coital stimulation received at the onset of estrus in gonadally intact rats [6]. Increasing numbers of penile intromissions received by these females did not result in as striking an abbreviation of estrus as had previously been reported for other species [3,13] or for ovariectomized, steroid-primed rats [19]. Gilman, Mercer, and Hitt [12] reported that fewer intromissions are required to initiate pseudopregnancy when females are allowed to regulate the temporal sequencing of intromissions received from stimulus males than when they are not able to "pace" intromissions. Accordingly, in the present study we compared the rates of estrus termination in animals allowed to pace their contact with stimulus males, animals receiving penile intromissions without pacing, and animals receiving mounts without penile intromissions. Paced coital stimulation greatly facilitated the rate at which estrus declined; this allowed us to examine brain indoleamine concentrations in animals exhibiting differing degrees of inhibition of lordosis behavior.

METHOD

Animals

Long-Evans hooded rats were purchased from Charles River Breeding Laboratories, Wilmington, MA. Females

(200–250 g) and males were housed 2/cage in suspended wire cages (17.5×20×25 cm). Food and water were available ad lib and lights were on between 2300 and 1100 hr.

Females were ovariectomized 3–4 weeks prior to the beginning of the experiments via bilateral flank incisions using ether as anesthetic. Sexually experienced male rats were used as stimulus animals.

Experimental Procedure

At the beginning of the experiment, ovariectomized female rats received a SC injection of estradiol benzoate (EB; 10 μg/rat in 0.1 ml sesame oil) at 0800 followed 48 hr later by an injection of P (500 μg/rat). Four hr after the P injection at 1200, animals were divided into three groups (n=7/group), and received differing coital stimulation with stimulus males: (1) 10 paced mounts-with-intromissions (Paced group); (2) 10 non-paced mounts-with-intromission (Non-Paced group); and (3) mounts without intromission (Mounts-only group). Females in the third group received 25 mounts, a number roughly equivalent to the total number (mounts with or without intromission) received in the other two groups, but were prevented from receiving cervical-vaginal stimulation by covering the vaginal opening with heavy cloth tape (vaginal mask) during the test.

The initial coital stimulation and subsequent tests of sexual receptivity were carried out in a room dimly lit by a 100 watt yellow light bulb. Animals were carried to this room for each test and returned to their home cages between tests. During the initial test period, the testing chamber was a glass aquarium (30×26×50 cm) containing corn chips divided by a removable Plexiglas barrier. In the lower half of this barrier was a small hole (3.5 cm dia.) through which the female could move from one side of the cage to the other. Experimental females were placed individually in this partitioned test cage for two 15 min sessions during the week preceding the initial test session; during these adaptation periods, all females moved readily from one side of the partition to the other. Prior to use as stimulus animals, males were placed in the chamber with sexually receptive stimulus females for 2 sessions during which they were trained to remain on one side of the partition by striking them on the nose when they attempted to pass through the partition. Using this or similar apparatus and test procedures [9,23], female rats will pace their sexual contact with stimulus males by entering the compartment containing the male immediately prior to a mount and leaving that compartment immediately following a mount. For animals receiving intromissions without temporal pacing or mounts only without intromission, the partition was removed and both males and females had free access to all parts of the test chamber.

Subsequent tests to assess the degree of sexual receptivity during the period following the initial contact with males occurred at 1500, 1800, 2100, and 2400. All females received 10 mounts from stimulus males in the undivided test chamber. Tests were approximately 20 min long. Vaginal masks were used on all animals to prevent further cervical-vaginal stimulation [15, 16, 30]. Immediately following the last test at 2400 hr, animals were decapitated, and brain tissue was removed and frozen for later indoleamine assay.

Lordotic responsiveness of the females was assessed by noting the occurrence of lordosis (concave arching of the back along with head and rump elevation) in response to mounts with pelvic thrusting by a stimulus male. The score derived was the lordosis quotient (LQ), the percent occurrence of lordosis.

Tissue Dissection and Indoleamine Assay

At the time of sacrifice, brains were quickly removed and placed immediately on dry ice for approximately 15 min. They were then placed on a glass plate over ice where dissection occurred while they were still frozen. Hypothalamus, brain stem, and frontal cortex were dissected using a small scalpel blade, and tissues were placed in 0.1 M perchloric acid (1 ml) and were frozen immediately on dry ice. Tubes were then stored in liquid nitrogen overnight. Tissues were thawed and disrupted by sonication the following morning. After sonication, samples were centrifuged (2200 g for 10 min) in the cold, and were refrozen until assay not more than 5 days later. Although some degradation of indoleamines occurs with storage, we estimated that 11–15% loss of 5-HT and 5-HIAA occurred within this 5 day period.

Tissue samples were obtained as follows: (1) hypothalamus (approximately 25 mg) was removed by making coronal cuts through the entire brain at the level of the optic chiasm and immediately anterior to the mammillary bodies, and by making similar parasagittal cuts at the lateral borders of the medial basal hypothalamus. The hypothalamic tissue was separated from the tissue dorsal to it by making a horizontal cut immediately below the anterior commissure; (2) a piece of frontal cortex (approximately 35 mg) was taken from tissue dorsal to the hypothalamus; and (3) the brain stem tissue was obtained from the remaining tissue beginning immediately posterior to the hypothalamus, extending from the mammillary bodies to the anterior border of the pons. After removal of the pons and cerebellum, the brain stem was bissected on the midline and either the left or right side was used (approximately 170 mg/hemi-brain stem).

Tissue levels of serotonin 5-HT and 5-HIAA were measured using a high-pressure liquid chromatograph (HPLC) equipped with an electrochemical detector (Bioanalytical Systems Model LC-50, West Lafayette, IN). The dimensions of the reverse phase column (Altex-Ultrasphere-ODS) were 4.6 mm × 15 cm, and the particle size was 5 μm. The working electrode was glassy carbon (TL-5, Bioanalytical Systems) with the sensitivity set at 1 nA/V and the working potential at 750 mV. The mobile phase was 13% methanol:0.1 M citric acid:0.1 M phosphate (dibasic) with 1 mM EDTA and 0.5 mM heptane sulphonic acid at pH 4.6. The flow rate through the column was 1.25 ml/min. Sensitivity of this method is 85 pg for 5-HT and 187 pg for 5-HIAA. This method will detect catecholamines as well as indoleamines, and has been validated previously (Menniti and Erskine, submitted).

On the day of assay, samples were thawed and were diluted 1:4 using additional perchloric acid. They were recentrifuged at 4°C for 20 min at 1600 g and were placed on ice until assay, at which time 50 μl of the supernatant was injected onto the HPLC. Protein content of the pellet in the original tube combined with that from the tube in which samples were diluted was measured using the method of Lowry [20]. Results are expressed as ng/mg protein.

Statistics

A two-way analysis of variance with repeated measures was used to examine overall effects of differing coital stimulation, using LQs from the four tests following the initial treatment. *Post hoc* comparisons were made using Scheffé's test. Indoleamine levels among groups for each tissue were compared with one-way analysis of variance.

TABLE 1

LEVELS OF SEROTONIN AND 5-HYDROXYINDOLEACETIC ACID IN INDICATED BRAIN REGIONS 12 HR AFTER COITAL STIMULATION OF OVARIECTOMIZED, STEROID-PRIMED RATS

Mating Stimulation	N		Hypothalamus	Brain Stem	Cortex
Mounts Only	7	5-HT	16.05 ± 1.10‡	6.12 ± 0.60§	5.76 ± 0.42
		5-HIAA	12.20 ± 1.46	4.42 ± 0.22§	3.35 ± 0.30
Non-paced Intromissions	7	5-HT	15.50 ± 0.70	8.27 ± 0.73†	5.80 ± 0.72
		5-HIAA	15.23 ± 2.58	7.18 ± 0.86*	3.32 ± 0.38
Paced Intromissions	7	5-HT	15.83 ± 1.34	7.86 ± 0.45†	5.77 ± 0.49
		5-HIAA	10.92 ± 1.36	7.07 ± 0.82*	3.52 ± 0.31

*Significantly different from Mounts Only group, *$p<0.05$; †$p<0.005$.
‡Mean ± S.E.M. Values are ng/mg protein.
§Based on 6 determinations.

RESULTS AND DISCUSSION

At the time of the initial test, 4 hr after the P injection, all animals showed LQs of 100 (Fig. 1). Paced and Non-Paced animals received, respectively, 24.0±4.0 (mean±SEM) and 19.7±2.1 mounts and mounts with intromission during the test. The mounts-only animals all received 25 mounts. Tests during which animals received paced stimulation were significantly longer (min from first to last mount = 17.10±2.83, ≤ 0.005) than tests in which non-paced (6.50±2.16 min) or mounts-only (6.29±1.22 min) coital stimulation occurred; thus the number of mounts/min was significantly lower in the Paced (2.01±0.44, $p \leq 0.05$) than in the Non-Paced (3.60±0.57) or the Mounts-only (4.91±1.01) groups.

During the subsequent four tests, the three groups of animals showed differential lordotic responsiveness overall (Groups: $F(92,18)=4.41$, $p \leq 0.05$; Fig. 1). Levels of lordotic responsiveness decreased over time as expected (Time: $F(3,54)=14.00$, $p \leq 0.001$), and the decline in receptivity was more rapid in Paced than in Non-Paced or Mounts-only animals, as reflected in a groups × time interaction, $F(6,54)=5.66$, ≤ 0.001. Animals receiving paced intromissions were significantly less receptive at 2400 hr than Non-Paced ($p \leq 0.001$) or Mounts-only ($p \leq 0.001$) animals, but did not differ significantly from the other two groups on any earlier test. Non-Paced animals did not differ at any time from Mounts-only animals.

These results confirm our observations in gonadally intact rats ([6]; Erskine and Baum, unpublished) that paced coital stimulation is effective in facilitating the process of estrus termination in this species, while a similar amount of non-paced cervical-vaginal stimulation has little effect. Other reports of a facilitatory effect of coital stimulation on estrus abbreviation in the rat [15,19] have not specified the types or amounts of coital stimulation received, but, with our procedures, non-paced intromissions are effective in facilitating estrus decline only if received in a number (25 intromissions) greatly exceeding that required for initiation of pseudopregnancy. While the present experiment did not determine the relative lengths of estrus in the three groups, it appears that temporally-appropriate coital stimulations shortens the length of the period of estrus, and thus may have profound effects on reproduction in this species.

5-HT concentrations in brain stem of both groups of animals receiving vaginal intromissions, regardless of the temporal pattern of those intromissions, were significantly higher than that seen in animals receiving mounts only, $F(2,17)=7.72$, $p \leq 0.005$; similar differences between the three groups were seen in brain stem content of 5-HIAA, $F(2,17)=4.29$, $p \leq 0.05$ (Table 1). Hypothalamic concentrations of both indoleamines were twice those seen in brain stem; however, levels of 5-HT and 5-HIAA did not differ among groups in this tissue. Concentrations of both compounds in cortex were uniformly low across groups. Although direct measurements of 5-HT turnover were not made, 5-HIAA content was used as an index of activity within serotonergic neurons since it has been suggested, on the basis of results obtained using the serotonin reuptake inhibitor, fluoxetine, that serotonin is converted to 5-HIAA

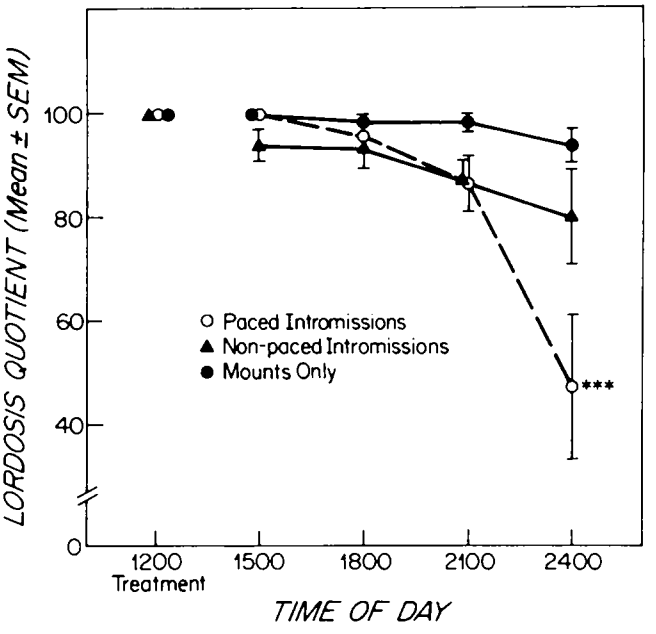

FIG. 1. Sexual receptivity (mean LQ ± SEM) exhibited by ovariectomized, steroid-primed rats given indicated coital stimulation at 1200 hr, and tested at 3 hr intervals for the following 12 hr. ***Significantly lower than LQs of the other two groups, $p \leq 0.001$.

after presynaptic reuptake [29], and that levels of 5-HIAA therefore reflect relative amounts of 5-HT released by nerve terminals.

The present results provide no support for the hypothesis that in rats the declining levels of sexual receptivity at the end of estrus result from increased serotonergic activity within hypothalamus or brain stem. No differences in tissue concentrations of 5-HT and 5-HIAA were seen in hypothalamus among groups showing differing levels of lordotic responsiveness immediately prior to sacrifice. In brain stem, similar elevations in 5-HT and 5-HIAA were seen in both groups receiving cervical-vaginal stimulation 12 hr earlier over levels seen in the mounts-only group; however, animals receiving paced intromissions were significantly less receptive than animals in either of the other two groups, whereas animals receiving mounts-only or non-paced intromissions did not differ in their levels of lordotic behavior at this time. Thus, the decrease in sexual receptivity brought about by coital stimulation is probably not due to increased serotonergic activity within brain regions examined.

These findings do not rule out the possibility that serotonergic neurons affect the intensity of lordosis during other times within the period of behavioral estrus, or that they are involved in the initiation of behavioral receptivity in response to ovarian hormones. In the present study a significant negative correlation (Pearson's $r=-0.48$, $p\leq 0.05$) was found between 5-HT levels in brain stem and lordosis quotients at 2400 hr. Since the brain stem dissection included serotonergic cell bodies within the raphe nuclei [27] as well as the mesencephalic central gray sites known to be facilitatory to lordosis [31], it is possible that influences of 5-HT on lordosis are mediated within this region.

It was somewhat surprising that there was not a significant correlation between 5-HT ($r=0.37$, ≤ 0.1) or 5-HIAA ($r=-0.01$) levels and lordotic behavior in hypothalamic tissue since localized implants of 5-HT receptor blockers in the anterior hypothalamus-medial preoptic area (AH-POA) stimulated lordosis [32], and infusion of 5-HT into AH-POA or the arcuate-ventromedial area of the hypothalamus [10] or intrahypothalamic implantation of a MAO inhibitor [21] decreased lordosis [10], suggesting that serotonergic terminals within the hypothalamus inhibit the expression of estrous behavior. If there are effects of ascending serotonergic projections from raphe to terminals within the hypothalamus on the expression of lordosis, they were not reflected in indoleamine concentrations at the time chosen for measurement.

The 5-HT receptor blocker, methysergide, facilitated lordosis in hormone-primed ovariectomized rats when it was implanted into amygdala and hippocampus [11], two areas which receive serotonergic inputs from brain stem [27]. It is possible that altered serotonergic activity at these non-hypothalamic sites is associated with the termination or inhibition of estrous behavior.

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