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NORADRENERGIC INFLUENCE ON THE
SEXUAL RECEPTIVITY OF FEMALE RATS
(Rattus norvegicus)

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NORADRENERGIC INFLUENCE ON THE SEXUAL RECEPTIVITY OF FEMALE RATS (*Rattus norvegicus*)

by

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ABSTRACT

NORADRENERGIC INFLUENCE ON THE
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Noradrenergic innervation of the medial preoptic area is believed to influence the timing of ovulation but studies have yet to elucidate its effect on sexual behavior. These experiments were designed to determine the effect of infusions of noradrenergic agents into the medial preoptic area (MPOA) on lordosis behavior in steroid hormone-treated ovariectomized rats.

In the first two experiments norepinephrine (NE) infusions into the MPOA reduced lordosis frequencies of estrogen-progesterone treated receptive rats. Norepinephrine doses of 2 ug or more per animal infused into the MPOA significantly reduced lordosis levels within five minutes. Lordosis quotients recovered to pre-infusion levels after twenty minutes. Infusions of 10 and 20 ug doses of NE suppressed lordosis levels 15 minutes after infusion. At the lowest inhibitory dose (2 ug/animal) simultaneous infusion of 5 ug/ul of the $alpha_2$-noradrenergic antagonist yohimbine blocked the reduction in lordosis resulting from NE infusion. Simultaneous infusion of noradrenergic antagonists phentolamine and propranolol did not block the inhibitory effects of 2 ug of norepineprine.

In a third experiment other noradrenergic agents were used to inhibit lordosis. In estrogen-progesterone treated animals the relative inhibitory effectiveness was: clonidine > epinephrine = norepinephrine > phenylephrine > methoxamine = isoproterenol. This hierarchy closely approximates the binding affinities of these agents to alpha$_2$-noradrenergic receptors. This correlation of inhibitory effectiveness and alpha$_2$-affinity may indicate that norepinephrine reduces lordosis levels via alpha$_2$-receptors.

In the fourth experiment bilateral infusions of six doses of norepinephrine (0.1 - 10 ug/animal) into the MPOA of estrogen-treated (0.5 ug EB injected for three days prior to testing) ovariectomized rats did not produce a significant dose-dependant increase in lordosis responding. A 20 ug NE dose, which resulted in some animal debilitation, was associated with a significant increase in lordosis. This increase was interpreted as a pharmacological and not a physiological effect. From these data it was concluded that NE does not directly enhance lordosis responding.

These data are consistent with the conclusion that the direct effect of norepinephrine infusions into the MPOA is inhibition of lordosis responding. There is some evidence that this inhibitory influence is mediated via alpha$_2$-noradrenergic receptors.

To Eunice

"Don't take it all so seriously."
Paul Engle to Kurt Vonnegut

"Nuthin's no big deal."
Charlie the Cook

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INTRODUCTION

Successful mammalian reproduction depends on the temporal coordination of ovulation and sperm deposition. This requires that the female allow the male vaginal access very near the time of ovulation. Various rodent species utilize different behavioral and physiological mechanisms to accomplish this close coordination. In some species, like the rabbit, copulation will initiate ovulation (induced ovulation, Sadleir, 1972) thus assuring that sperm and egg are present in the oviducts at the same time. However, in the female rat ovulation takes place on a four or five day cycle with or without mating (spontaneous ovulation, Sadleir, 1972). Something must cue the female rat's neural system to initiate behaviors which will allow the male to inseminate her at or about the time of ovulation.

The time in the rat's cycle just after ovulation is called estrus and the behaviors of a female at this time are estrous behaviors. Estrous behaviors include those actions by the female to attract the male and her response to the male. If the female's response to the male's mount increases the likelihood he will achieve intromission the behavior is called receptive behavior. The most striking component of receptivity in rats is lordosis. Lordosis consists of a ventral flexion of the back and concomitant lifting of the perineal region. This allows the male penile access to the vagina. Lordosis is thus an important aspect of estrous behavior.

Removal of the ovaries eliminates estrous behaviors. The ovaries are the site of oocyte development and the source of quantities of the steroid hormones estrogen and progesterone. No estrous behavior is seen when the ovaries are removed before maximal progesterone release, but receptivity is seen if the ovaries are not removed until after they release progesterone (Powers, 1970). In ovariectomized rats administration of estrogen alone (Davidson et al., 1968) or in combination with progesterone can restore all observable receptive behaviors (Boling & Blandau, 1939; Beach, 1942; Edwards, Whalen & Nadler, 1968). It is apparently the estrogen and progesterone from the ovaries and not the act of ovulation which control estrous behavior. Normally the lordosis response is elicited by palpation of the flanks and penile stimulation of the perineum by the male (Boling, Blandau and Young, 1941; Edwards, Whalen & Nadler, 1968; Kow & Pfaff, 1976). Without estrogen the female will not respond to such stimulation with a lordosis (Edwards, Whalen & Nadler, 1968; Kow & Pfaff, 1976). It appears that estrogen alters the response pattern to this specific somatosensory input. If progesterone is also administered less estrogen is necessary to attain similar lordosis response levels (Boling & Blandau, 1939). Thus the ovarian hormones estrogen and progesterone act synergistically to induce a change in somatosensory responsiveness which is responsible for receptivity.

Any change in somatosensory responsiveness would be mediated by the nervous system. Though estrogen can act on peripheral receptive fields (Komisaruk, Adler & Hutchinson, 1972; Bereiter & Barker, 1980) estrogen

does not alter lordosis reponsiveness in animals with a transection of the upper spinal cord (Hart, 1969). Thus the effects of the ovarian hormones on receptivity are most likely manifested in the brain. The ovarian hormones are bound in a number of brain regions including the medial preoptic area (MPOA). Cells from the medial preoptic area (MPOA) sequester radiolabelled estrogen in the cytosol (Stumpf, 1968; Pfaff, 1968; Zigmond & McEwen, 1970; Marrone & Feder, 1977; Blaustein et al. 1979) and in the nucleus (Blaustein et al., 1980; Leiberburg et al., 1980). Estrogen also alters the firing patterns of medial preoptic neurons (Bueno & Pfaff, 1978; Leung et al., 1981). Estrogen will induce formation of progestin receptors in preoptic tissue (Moguilewsky & Raynaud, 1977; MacLusky & McEwen, 1978; Moguilewsky & Raynaud, 1979; Blaustein & Feder, 1980; Blaustein, Ryer & Feder, 1980; Parsons et al., 1980) indicating that this area could mediate the synergistic effects of estrogen and progesterone on estrous behavior. This all could be attributed to the gonadotropin-controlling role of the MPOA. However, direct implantation of estrogen into the MPOA can induce receptivity in ovariectomized animals (Lisk, 1962) and progesterone implantation here can increase female receptivity in estrogen-treated males and females (Powers, 1972; Ward et al., 1975; Ward, Franck & Crowley, 1977). The preoptic enhancement of lordosis after direct steroidal implantation indicates the capacity for these hormones to influence receptivity by affecting preoptic activity.

Ovarian hormones, particularly estrogen, interact with brain regions such as the MPOA to somehow elicit receptivity. Estrogen seems to act on these functions with a long latency (Parsons et al., 1980). This latency may reflect a mechanism which requires some time to exhibit maximal estrogen effect, such as protein synthesis. Many researchers contend that estrogen is translocated into the nucleus where it binds to the chromatin (O'Malley & Schrader, 1976; Liebl & Spona, 1982). Once attached to the non-histones surrounding the DNA estrogen may alter protein synthesis (King et al., 1979; Rainbow, Davis & McEwen, 1980; Parsons & McEwen, 1981). Estrogen may act on preoptic neurons to increase their capacity to synthesize or respond to other neurochemicals in much the same manner. Estrogen is already known to affect synthesis, enzyme activity, receptor parameters and turnover of a number of putative neurotransmitters. Estrogen affects the activity of neurotransmitter-controlling enzymes acetylcholinesterase, monoamine oxidase (Luine, Khylchevskaya & McEwen, 1975), choline acetyltransferase (Muth, Crowley & Jacobowitz, 1980) and tyrosine hydroxylase (Beattie, Rogers & Soyka, 1972). Estrogen also affects preoptic-hypothalamic serotonin receptors (Biegon, Berkowitz and Samuel, 1980), and acetylcholine receptor number (Dohanich et al., 1981).

Medial preoptic and hypothalamic noradrenergic systems are particularly sensitive to estrogen. The neuronal cell bodies which synthesize norepinephrine also sequester estrogen (Heritage et al., 1980). These same neurons send projections to the MPOA (Dahlstrom & Fuxe, 1965; McBride & Sutin, 1976). After ovariectomy norepinephrine

turnover rates increase in the MPOA and hypothalamus (Anton-Tay, Pelham & Wurtman, 1969; Advis, McCann & Negro-Vilar, 1980). Estrogen treatment will reduce this NE turnover in the MPOA of ovariectomized rats (Lofstrom et al., 1977; Hohn & Wuttke, 1978; Honma & Wuttke, 1980). Norepinephrine turnover is low following estrus and rises to a peak during proestrus (Wise, Rance & Barraclough, 1981). Activity of the preoptic noradrenergic system is heavily influenced by the ovarian hormones.

The cyclic release of estrogen and progesterone also controls ovulation. The timing of ovulation is controlled by the release of gonadotropins from the anterior pituitary (see Schwartz, 1973 for review). In rats a surge of luteinizing hormone (LH) immediately precedes ovulation (Short, 1972). Release of LH is, in turn, controlled by bursts of a medial preoptic decapeptide called gonadotropin releasing hormone (GnRH; see Short, 1972; Sarkar, 1976; Vale, Rivier & Brown, 1977). Noradrenergic innervation of the MPOA plays a major role in controlling the synthesis and release of GnRH. Review of a number of studies indicates that norepinephrine acts to enhance plasma LH levels (see Weiner & Ganong, 1978 for review). Microinfusion of norepinephrine into the ventricles increases plasma LH levels in animals treated with estrogen and progesterone (Krieg & Sawyer, 1976; Gallo & Drouva, 1979). This hormonal combination may mimic the steroidal environment during proestrus. Since norepinephrine turnover (Wise, Rance & Barraclough, 1981) and levels (Stefano & Donoso, 1967) increase during proestrus, the proestrous surge in NE would appear to stimulate the release of

pituitary stores of LH. Norepinephrine may stimulate the LH surge via alpha receptors. Specific alpha-noradrenergic blocking agents phenoxybenzamine and phentolamine disrupted pulsatile LH release (Weick, 1978). An alpha-adrenergic agonist, epinephrine, stimulates the release of LH (Vijayen & McCann, 1978) and the artificial alpha-agonist clonidine restores LH surges in norepinephrine-depleted rats (Estes, Simpkins & Kalra, 1982). One conclusion from these data would be that norepinephrine acts on alpha-noradrenergic receptors to increase either the number or amplitude of LH surges. Norepinephrine is ineffective or even inhibits LH surges in non-hormonally treated animals (Drouva & Gallo, 1976; Leung et al., 1982) indicating the degree of noradrenergic-hormonal interaction in the gonadotropin system. The complexity of this interaction may be expressed in very specific localization of function since the effects on LH surges varies depending on whether NE is infused into one brain nucleus or one adjacent to it (Parvizi & Ellendorff, 1982). The question of preoptic norepinephrine's role in control of ovulation is not solved, but there are strong indications that a pre-ovulatory increase in NE turnover stimulates release of GnRH by MPOA neurosecretory cells which induces a pre-ovulatory LH surge.

For all the importance of preoptic norepinephrine in control of ovulation little is understood of its role in control of the accompanying receptivity. Preoptic NE turnover is high before behavioral estrus and low after (Wise, Rance & Barraclough, 1981). It is not known whether NE turnover remains high throughout estrus or drops

after the massive proestrous surge.

Attempts to artificially manipulate preoptic and hypothalamic levels of NE have yielded conflicting results. Administration of 6-hydroxydopamine (6-OHDA), which selectively destroys catecholaminergic neurons and thus reduces catecholamine transmission, increases lordosis behavior (Herndon et al., 1978). However, one of these researchers also found that electrolytic lesion of noradrenergic neuronal cell bodies in the brainstem would disrupt estrous behavior and that amphetamines, which release NE, could partially restore receptivity. However, in non-lesioned animals amphetamines decreased female sexual behavior (Meyerson, 1968; Eliasson, Michanek & Meyerson, 1977). Increased lordosis levels have been demonstrated following systemic treatments of alpha-methyl-p-tyrosine, which reduces catecholaminergic synthesis, and with the alpha-noradrenergic antagonists piperoxane and yohimbine (Everitt et al., 1975; Caggiula et al., 1979). Use of the alpha-agonist clonidine systemically had a striking inhibitory effect on receptivity but no facilitative effect was evidenced (Davis & Kohl, 1977). Some of the discrepancies in these results may be due to the use of systemic injections rather than direct intracerebral infusions. However, direct infusions of noradrenergic agents into the MPOA beta-agonists were found to enhance lordosis/mount ratios in one study (Foreman & Moss, 1978), while infusion of a beta-antagonist into the MPOA facilitated lordosis in another study (Ward et al., 1975). Because of the different experimental manipulations and behavioral measures used it is difficult to conclude what role preoptic norepinephrine plays in control of rodent receptivity.

It is understood that both the MPOA and NE are important in the control of fertility, but because of the contradictory nature of the behavioral results little can be concluded about NE's contribution to estrous behaviors. Because norepinephrine acts both centrally and peripherally, systemic manipulations of this neurotransmitter may not be affecting only central mechanisms. The most direct way to test the central effects of NE on receptive behaviors is to infuse norepinephrine and/or noradrenergic agents directly into relevant brain regions, such as the MPOA. These experiments are designed to do just that; to concentrate on infusion of NE into the MPOA and its effects on lordosis in ovariectomized rats in various receptivity states. The animals' levels of receptivity are manipulated by injections, in various combinations, of estrogen and progesterone. No experiments to date have rigorously explored the affects of a range of NE doses infused directly into the brain on lordosis behavior. It is hoped that these experiments will identify what effect an exogenous pulse of norepinephrine into the MPOA will have on this discrete, important and hormonally-controlled behavior.

BACKGROUND

The Introduction implicates noradrenergic input to the medial preoptic area in the coordination of fertility and receptivity of female rats. This section will attempt to expand on this theme and fill in some important background material. Estrogen is an ovarian steroid which is responsible for the timing of ovulation and the occurrence of estrous behavior. The discussion will begin by looking at possible sites of estrogen's action. One possible site of estrogen action is the medial preoptic area (MPOA). An overview of the neuroanatomy of the MPOA may prove helpful in understanding its role in estrous behavior and endocrine control. Noradrenergic neurons provide a major afferent innervation to the MPOA. It is an input known to affect the release of several pituitary hormones. Some background is given of norepinephrine-like chemicals called catecholamines. An understanding of general catecholaminergic metabolic pathways and mechanisms is important for comprehension of the experiments to follow. After a discussion of noradrenergic mechanisms this paper will focus on norepinephrine's role in the MPOA and its association with ovulation. Finally, evidence of norepinephrine's effects on estrous behavior will be reviewed. These components should help elucidate the reasons for the following experiments.

Sites of Estrogen Action

This segment will deal with those sites of estrogen binding which have been implicated in the control of estrous behavior. While estrogen binds to uterine tissue (Pavlik & Coulson, 1976; Marrone & Feder, 1977; Liebl & Spona, 1982) few people recognize a major role for the uterus in the control of estrous behavior in the rat. There is, however, evidence that estrogen can act in the periphery to enlarge trigeminal (Bereiter & Barker, 1980) and genital sensory fields (Komisaruk, Adler & Hutchison, 1972). This could be due to estrogen's action on the dermal sensory receptors themselves or may be mediated by an action on the spinal ganglia or cord. While some animals with high spinal cuts are capable of demonstrating lordosis reflexes, these responses are not altered by ovarian hormone treatment (Hart, 1969) as is seen in intact animals (Boling & Blandau, 1939; Beach, 1942; Davidson et. al., 1968). Thus the steroid hormones probably affect receptivity by acting on the brain. Two major studies measured brain sites which bind tritiated estradiol. Pfaff found heavy estradiol binding in the following basal areas: prepiriform cortex, ventromedial hypothalamus, olfactory tubercle, septum, amygdala and the medial preoptic area (Pfaff, 1968). Stumpf, using a dry-mount technique, found heavy estradiol binding in the arcuate nucleus, lateral and caudo-lateral ventromedial hypothalamus, cells ventral to the magnocellular part of the paraventricular nucleus, the preoptic region of the periventricular nucleus, lateral septal nuclei, nucleus accumbens, interstitial nucleus of the stria terminalis, subfornical organ, triangular septal nucleus, suprachiasmatic nucleus of

the preoptic area and also the medial preoptic nucleus (Stumpf, 1968). Estrogen is important in a number of behaviors and physiological functions. Perhaps this is why it binds at so many diverse brain sites. But further evidence indicates that some of these areas, as the medial preoptic area (MPOA), also will bind tritiated progestins if animals are pre-treated with estrogen (Moguilewsky & Raynaud, 1977; Marrone & Feder, 1977; MacLusky & McEwen, 1978; Blaustein & Feder, 1980; Parsons et al., 1980). The MPOA is especially suited to mediate the synergistic action of estrogen and progesterone on both ovulation and estrous behavior.

Medial Preoptic Area Neuroanatomy

The preoptic area has an old phyletic history. Cells aggregated into discrete nuclei are definitely distinguishable in amphibians (Mautner, 1964) and more diffuse cell collections are seen in similar post-olfactory areas in fish (Jorgensen & Larsen, 1967). Amphibians have preoptic neurons which contain gonadotropin-releasing peptides (Zambrano & DeRobertis, 1968). The preoptic recess organ of amphibians shows noradrenergic histofluorescence (Rao & Hartig, 1974; Hanke, 1976). Some preoptic cells in amphibians concentrate radiolabelled steroids (Kelley, Morrell & Pfaff, 1975; Morrell, Kelley & Pfaff, 1975). If the evidence from extant species holds for amphibian ancestors, this brain region may have monitored blood hormone levels from very early. It may not be too wild a speculation that later connections and associations were partially to exert some neural control on these original endocrine systems.

In mammals the preoptic region is closely associated anatomically and functionally with the hypothalamus, but has long been thought to derive from unevaginated telencephalon (His, 1893). Gurdjian located the preoptic region by indicating that it lies just caudal to the septum and tuberculum olfactorium and just in front of the hypothalamus with which it is said to "merge insensibly" (Gurdjian, 1927). It is bordered dorsally by the anterior commissure, ventrally by the optic chiasm and surrounds the third ventricle. Gurdjian described a periventricular preoptic nucleus with five layers (1927). Bleier and her colleagues indicate a periventricular preoptic nucleus (Bleier, Cohn & Siggelkow, 1979) which actually corresponds to an area Loo calls the nucleus preopticus medianus, which consists of small, granular spindle-shaped cells (Loo, 1931). What Loo refers to as the nucleus preopticus periventricularis (1931) corresponds to Bleier's medial preoptic nucleus (1979). The principle medial preoptic nucleus was split by Gurdjian into a well-defined lateral portion made up of medium-sized cells and paler more medial cells which are continuous with the anterior hypothalamus (1929).

An interesting and probably important characteristic of preoptic neuroanatomy is the reciprocal nature of afferent and efferent connections to and from this area. For example, degeneration techniques demonstrate that olfactory tubercle cells innervate the preoptic area and vice versa (Mizuno, Clemente & Sutherland, 1969). Golgi studies show lateral septal axons entering the rostral preoptic area (Valverde, 1963) while Conrad and Pfaff saw projections from the POA to the lateral

but not the medial septum (Conrad & Pfaff, 1975). One of these reciprocal connections which is significant in endocrine control is between the MPOA and the arcuate nucleus. Dyer has demonstrated direct antidromic invasion in 41% of preoptic/anterior hypothalamic neurons tested after ventromedial/arcuate electrical stimulation and claims both inhibitory and excitatory input from the ventromedial/arcuate area to the preoptic area (Dyer, 1974).

Reciprocal connections between MPOA cells and noradrenergic cell bodies in the locus coeruleus are of great importance for endocrine and possibly behavioral control. Cells in the locus coeruleus are known to bind tritiated-estradiol (Heritage et al., 1980). Fink-Heimer staining and radiolabelled amino acid procedures together demonstrated locus coeruleus projections which travel through the mesencephalic gray to split up into dorsal and ventral fascicles (McBride & Sutin, 1976). The dorsal fascicle continues to intralaminar thalamic nuclei whereas the ventral pathway passes the medial lateral hypothalamus to terminate in the POA and septum. Lesioning or cutting of the more lateral medial forebrain bundle, but not the more medial tracts, will result in a drop in preoptic catecholamine levels (Palkovitz et al., 1977). Horseradish peroxidase injected into the preoptic area is carried back to locus coeruleus cell bodies (Berk & Finkelstein, 1981). Conrad and Pfaff found efferents from the POA to areas lateral and anterior to the locus coeruleus (1976). More specifically, Swanson found medial preoptic efferents projecting into the central gray near the locus coeruleus (Swanson, 1976). This reciprocal connection between the MPOA and

noradrenergic cell bodies of the locus coeruleus may indicate that not only does norepinephrine enter the MPOA, but that the MPOA may modulate its own input from these neurons. Estrogen's binding to both of these areas which are interconnected provides further evidence that the noradrenergic system is important in ovulation and/or estrous behavior.

Medial Preoptic Function

The medial preoptic area was seen above to be a major binding site for tritiated estradiol. The preoptic area, like the hypothalamus immediately caudal, is highly vascularized (Ambach, & Palkovitz, 1979). This offers it the opportunity to monitor blood titers of steroids and other ciculating hormones. This blood-monitoring capacity plus its proximity to and innervation from several olfactory sensory systems, makes the preoptic area an important endocrine control region. Neurons in the MPOA seem to control the release of several anterior pituitary hormones. The corticotropin releasing factor, which controls the pituitary release of adrenocorticotropic hormone (ACTH, and possibly β-endorphin), is modified by MPOA manipulation (see Van Loon, 1973; Weiner & Ganong, 1978 for reviews). The tripeptide releasing hormone for thyrotropin stimulating hormone (TSH), thyrotropin releasing hormone (TRH), also affects neural electrical activity in the MPOA (Salzman & Beckman, 1980) which is one suggested site for TRH synthesis (see Krulich et al., 1974 for review). There is also some evidence that noradrenergic activity in this area controls growth hormone release (see Chambers & Brown, 1976; Martin, 1976; Weiner & Ganong, 1978) and that

alpha-noradrenergic receptors mediate this control (Durand, 1978; Eriksson, Eden & Modigh, 1981). But, by far the most extensively studied of the preoptic neuropeptides is gonadotropin-releasing hormone (GnRH), which controls the anterior pituitary release of follicle stimulating hormone (FSH) and luteinizing hormone (LH; Sarkar et al., 1976). This decapeptide has been identified in the MPOA (Barry & DuBois, 1973; see Knigge et al., 1980 for review). Some authors contend it is transported to capillary beds near the arcuate region where it is released into the portal system in bursts (Sarkar et al., 1976; Ojeda, Negro-Vilar & McCann, 1982). Estrogen is very important in the feedback control of GnRH synthesis and release (Gay & Midgely, 1966). Several neurotransmitter systems have been implicated in the control of this peptide (see Weiner & Ganong, 1978 for review). However, norepinephrine is the neurotransmitter most often cited as interacting with estrogen to control LH release (Kalra & McCann, 1973; Tima & Flerko, 1974; Krieg & Sawyer, 1976; Estes, Simpkins & Kalra, 1982; Leung et al., 1982).

Other important functions have been attributed to the preoptic area. Some of these functions may be correlated with the occurrence of ovulation and estrous behaviors. For example, during sexual activity several autonomic parameters are known to vary, such as blood pressure, temperature, vasodilation, salivary secretions, and galvanic skin response (see Zuckerman, 1971 for review). The preoptic area has been associated with control of autonomic functions for some time (Hess, 1954 & 1957). It is believed to be a center for thermoregulation and blood pressure control (see Boulant, 1980 for review). Cooling or heating the

preoptic area will alter an animal's thermoregulatory behavior (Freeman & Davis, 1959; Roberts & Mooney, 1974). Norepinephrine (NE) is an important neurotransmitter in mediating this effect. Infusions of 1 ug NE into this area will result in a transient hyperthermia in monkeys (Myers & Yaksh, 1969) and infusions of NE will have the same result in rats (Veale & Whishaw, 1976; Day, Willoughby & Geffen, 1979). Such infusions can elicit changes in heart rate, brain temperature and blood pressure in baboons (Toivola & Gale, 1970). Noradrenergic innervation of the MPOA appears to control a number of autonomic functions (see Myers, 1980 for review). It is perhaps significant that several of these autonomic functions are greatly altered during sexual activity.

Catecholamines: Synthesis, Metabolism, Release and Receptors

A better understanding of norepinephrine, and norepinephrine-like agents, may be helpful in determining NE's role in control of ovulation and receptivity. Norepinephrine belongs to a group of neuroactive chemicals called catecholamines. Catechol refers to the 3,4-dihydroxyphenyl nucleus to which variously constructed amine groups are attached to give the three main catecholamines: dopamine, norepinephrine and epinephrine, in that metabolic order (Nagatsu, 1973). The liver hydroxylates phenylalanine to make the precursor for the catecholamines - tyrosine (Udenfriend, 1966b). Tyrosine is hydroxylated to dihydroxyphenylalanine (DOPA) in sympathetic nerves, heart, adrenal medulla and brain (Udenfriend, 1966b). Some of these tissues contain other enzymes such as DOPA-decarboxylase to turn DOPA into dopamine,

dopamine beta-hydroxylase to convert dopamine to norepinephrine and phenylethylamine-N-methyltransferase (PNMT) to methylate norepinephrine's nitrogen to make it epinephrine (Udenfriend, 1966a). Catecholamines are normally broken down by the replacement of an hydroxyl group by an ester, which is accomplished by catechol-o-methyltransferase (COMT; Axelrod, 1957). The resulting metanephrine and normetanephrine are further reduced by mitochondrial monoamine oxidase (MAO) to vanillylmandelic acid or 4-hydroxy-3-methoxy phenylglycol (Blaschko & Schlossmann, 1936). This is only the first discovered degradative pathway. There are other pathways which result in numerous other catecholaminergic metabolites.

The catecholamines are found in specific tissues such as adrenal medulla, brain and peripheral nerves. Most of the early work on the metabolism of the catecholamines dealt with chromaffin cells of the adrenal gland. Much of the work on the role of the catecholamines as neurotransmitters still deals with peripheral organs such as the heart and aorta, vascular beds, vas deferens and other smooth muscle. In these preparations and in brain tissue preparations it is seen that norepinephrine is axonally transported to synaptic terminals where it eventually is sequestered in small vesicles (Smith, 1973; Smith, 1979). These small vesicles release the NE either by exocytosis or by forming tight junction with the plasma membrane (Smith, 1979). Once released the catecholamines can be taken back up by the presynaptic neuron, broken down by the synaptic enzymes, move into extrasynaptic space or attach to specific or unspecific receptors on other cells (Langer,

1974). Noradrenergic agents at the synapse may be able to attach to pre- or post-synaptic receptors (Berthelsen & Pettinger, 1978). The effects and nature of these noradrenergic or adrenergic receptors will be discussed. If the catecholamines are taken back into the presynapse they are, most likely, catabolized by mitochondrial MAO (Langer, 1974; Mandel, Mack & Goridis, 1975). If they remain in the synaptic cleft they are broken down by post-synaptic or extra-synaptic COMT (see Langer, 1974 for review). Any neurotransmitter would not remain intact for long in the synaptic cleft.

Neurotransmitters are released in quanta into the synaptic cleft to attach to and affect highly specific protein receptors. In the case of the catecholamines there are a number of different proteins which bind these transmitters and some of these protein receptors may exist in multiple states. The action any catehcholamine will have will depend on which receptors it binds to and possibly on when it binds to that specific receptor. Ahlquist (1948) was the first to suggest that different catecholaminergic receptors existed. He referred to the two types of receptors as alpha and beta. With the refinement of radioligand techniques, chemicals were found which bind with very different affinities to binding sites within each of these classifications. Thus it was necessary to introduce $alpha_1$, $alpha_2$, $beta_1$, and $beta_2$ subtypes. Since radioligand assays could distinguish different characteristics between these subtypes it was thought that they might play different functional roles. Based on studies using cardiac or smooth muscle preparations Berthelsen and Pettinger suggested

that alpha$_1$-receptors were mostly post-synaptic and that alpha$_2$ were presynaptic and probably acted like autoreceptors, inhibiting norepinephrine's release (Langer, 1974; Berthelsen & Pettinger, 1978). The generalizations about the nature of pre- and post-synaptic noradrenergic receptors may not apply to the central nervous system. Alpha$_2$-receptors do exist on locus coeruleus cell bodies (Aghajanian & VanderMaelen, 1982), which are probably autoreceptors. However, alpha$_2$-receptors also exist in many regions of the brain where alpha$_1$-receptors are not found (Young & Kuhar, 1979; Young & Kuhar, 1981; Leibowitz et al., 1982). The MPOA is one site of alpha$_2$-binding (Leibowitz et al., 1982). Injections of 6-OHDA which should destroy presynaptic structures of norepinephrine neurons, fails to decrease alpha$_2$-receptors in cortex and other brain areas (U'Prichard et al., 1979; U'Prichard et al., 1980). These alpha$_2$-receptors do not seem to be all presynaptic in the brain. It is such alpha$_2$-receptors which appear to be responsible for blood pressure control in the preoptic area (Struyker-Boudier et al., 1974) and are also involved in the control of GnRH, GH and ACTH release (Durand, Martin & Brezeau, 1978; Eriksson, Eden & Modigh, 1981; Estes, Simpkins & Kalra, 1982; Leung et al., 1982).

Almost all of the catecholaminergic neurons known have their cell bodies in a few brain areas. Dopamine cell bodies have been identified in the tuber cinereum with processes into the neurohypophysis or infundibulum thus they are called tuberoinfundibular neurons (nucleus A12 according to Dahlstrom & Fuxe, 1964; see also Lindvall & Bjorklund, 1978; Moore & Bloom, 1978), some are found in or near the substantia

nigra (cell groups A8 and A9) with processes to the striatum (Dahlstrom & Fuxe, 1964; Dahlstrom & Fuxe, 1965) and in a midbrain cell group called A10 (Ungerstedt, 1971). The locus coeruleus has already been mentioned as a site of norepinephrine-containing cell bodies (A6 cell group). Cell bodies in the dorsal medullary (A2), medullary (A1 & A3) and pontine (A5 & A7) parts of the lateral tegmental noradrenergic system also contribute to this system with processes which ascend near the central tegmental tract (ventral noradrenergic bundle) or a part of the dorsal tegmental tract (dorsal noradrenergic bundle). These two pathways eventually join the medial forebrain bundle in the caudal hypothalamus. Another route for pontine and medullary noradrenergic axons runs near the third ventricle along with some periventricular dopamine neurons (see Leibowitz, 1980). It is the lateral tegmental and dorsal medullary cell groups (A1, A2, A5 and A7) which widely but unevenly innervate the hypothalamus. Less is known of the origin and terminations of epinephrine neurons. Assays have found PNMT in hypothalamic sites (Hokfelt et al., 1974). Cell bodies for such epinephrine synthesizing neurons are only known to exist in two cell groups (C1 and C2) lying near NE medullary groups A1 and A2 (see Leibowitz, 1980). All of the catecholaminergic neuronal nuclei send processes to the various hypothalamic nuclei and may in turn receive hypothalamic efferents back from those innervated areas (see above).

Norepinephrine and Ovulation

Medial preoptic norepinephrine has already been linked to control of autonomic functions and pituitary hormone release which take place in association with ovulation. There is good evidence that preoptic norepinephrine plays a key role in controlling GnRH release and thereby ovulation itself. An example of this was evidence that preoptic NE levels were elevated, along with plasma LH levels, following castration (Hohn & Wuttke, 1978; Honma & Wuttke, 1980). A number of studies attempted to correlate high NE levels and/or turnover in the MPOA with high LH levels (Anton-Tay, Pelham & Wurtman, 1969; Kalra & McCann, 1973; Kalra & McCann, 1974; Honma & Wuttke, 1980). Norepinephrine was discovered to peak in the afternoon of proestrus before the LH peak (Wise, Rance & Barraclough, 1981). Ovulation and LH surges can be induced in hormone-treated animals with intracerebral infusions of NE or injections of noradrenergic-enhancing agents (Tima & Flerko, 1974; Krieg & Sawyer, 1976; Gallo & Drouva, 1979). On the other hand, drugs which interfere with noradrenergic function will block LH surges and ovulation. Dopamine-β-hydroxylase (DBH) inhibitors given before the critical period of proestrous blocks the LH surge (Kalra & McCann, 1974; Drouva & Gallo, 1976). Luteinizing hormone surges induced by electrical stimulation of the preoptic region were blocked by a DBH inhibitor and this effect was overcome by adding a norepinephrine precursor which bypasses the DBH enzyme, dihydroxyphenylserine (DOPS; Kalra & McCann, 1973). Blocking normal norepinephrine synthesis halted natural and induced LH surges and so did blockade of alpha-noradrenergic receptors.

Weick found that two alpha-noradrenergic antagonists, phenoxybenzamine and phentolamine, blocked pulsatile discharges of LH for 65-120 minutes (Weick, 1978) and that alpha-adrenergic agonists epinephrine and clonidine were capable of inducing LH surges (Vijayen & McCann, 1978; Estes, Simpkins & Kalra, 1982). There is some question as to where norepinephrine would have its stimulating effect on LH release. Certainly NE terminals are found in the MPOA and NE affects firing rates of MPOA neurons (Leung et al., 1981), but ventricular injection of NE also increases median eminence neural activity as well (Krieg & Sawyer, 1976). Thus NE may act on preoptic neurons to increase GnRH synthesis or transport or on median eminence cells to increase GnRH release or both. But stimulation of central alpha-noradrenergic receptors seems an important component of gonadotropin-initiated ovulation.

The Medial Preoptic Area and Receptivity

The role of the MPOA in control of ovulation and of many physiological parameters associated with reproduction has already been elaborated. This basal brain region's role in the behavior associated with reproduction is less well understood. It is the site of gross neuroanatomical differences between males and females in several species (Gorski et al., 1978; Gorski et al., 1980; Jacobsen & Gorski, 1981; Byne, Bleier & Siggelkow, 1982). These anatomical distinctions are altered by perinatal hormone treatments (Jacobsen et al., 1980). Lesion of this region results in decrements in male sexual behaviors (Heimer & Larson, 1966/67; Christensen, Nance & Gorski, 1977). However, studies

to date have found no loss of female receptivity as a result of preoptic lesions (Singer, 1968; Powers & Valenstein, 1972). Electrical stimulation of this area results in inhibition of hormone induced receptivity (Pfaff & Sakuma, 1979). These data have been cited by others to show that the MPOA is an inhibitory center with respect to female reproductive behaviors. However, categorization of the MPOA as an "inhibitory center" for receptivity may be simplistic and incorrect. The MPOA is known to sequester estrogen (Pfaff, 1968; Stumpf, 1968; Zigmond & McEwen, 1970; Marrone & Feder, 1977; Blaustein et al., 1979; Blaustein et al., 1980) and to produce progestin-receptors in response to estrogen (Moguilewsky & Raynaud, 1977; MacLusky & McEwen, 1978; Blaustein, Ryer & Feder, 1980; Parsons et al., 1980). Therefore the MPOA has the substrate for ovarian hormone action. Implanting the ovarian hormones into the MPOA can activate sexual behavior in unreceptive females. Estrogen implants here in ovariectomized animals resulted in receptivity increases (Lisk, 1962; Yanase & Gorski, 1976). Implants of progesterone in this area of estrogen-treated males and females will result in augmented receptivity (Powers, 1972; Ward et al., 1975; Ward, Franck & Crowley, 1977). It is difficult to reconcile the positive influence of preoptically implanted ovarian hormones with postulates which contend that the MPOA is an inhibitory area for reproductive behaviors.

The medial preoptic area has been shown to be important in the control of many physiological functions associated with ovulation and receptivity. The location of the MPOA, at a crossroad between the

olfactory forebrain and the hypothalamus, and its reciprocal innervations from areas which bind ovarian hormones make it a prime candidate for the mediation of sensory input by steroids and control of the motor responses which make up receptive behaviors. The assignation "inhibitory center" adds little to our understanding of the actual role of medial preoptic cells in control of ovulation and female receptivity.

Norepinephrine and Receptivity

The MPOA is important in the control of many reproductive functions, many of these functions are controlled or influenced by noradrenergic activity. However, its role in estrous behavior is still unknown. A female rat's behavior at estrus is distinct in many ways from that at other times in its cycle (see Beach, 1942 for review). In rats the male's mount will result in a ventral flexion of the back by the female or lordosis, which allows the male vaginal access. The lordosis response, to flank palpation and penile thrusting by the male, occurs almost immediately after the sensory stimuli (see Kow & Pfaff, 1976).

Ovarian hormones are necessary, over the long term, for the occurrence of lordosis. However, few researchers would suggest that estrogen and progesterone are released from neural stores in response to the immediate somatosensory input of the male's mount. These hormones are thought to alter activity of endogenous transmitter systems to change the way a female responds to a male mount (see above).

Norepinephrine is one endogenous central neurotransmitter which is affected by ovarian hormone levels. The cell bodies of noradrenergic neurons bind estrogen (Heritage et al., 1980). There is a striking increase in norepinephrine levels and turnover after ovariectomy, when animals would be unreceptive (Anton-Tay, Pelham & Wuttke, 1969; Hohn & Wuttke, 1978; Honma & Wuttke, 1980). Treatment of these animals with estrogen will reduce their preoptic noradrenergic turnover levels (Anton-Tay, Pelham & Wurtman, 1969). In cycling animals preoptic norepinephrine levels are relatively low after estrus and increase to a peak on the afternoon of proestrus (Wise, Rance & Barraclough, 1981). This increase in turnover rates apparently precedes the LH surge, which it may help initiate. Thus preoptic norepinephrine peaks before the onset of estrus. Because most studies concentrate on norepinephrine turnover in relation to the LH surge, there is no direct evidence of preoptic noradrenergic turnover during behavioral estrus. Most such attempts instead study norepinephrine parameter changes as a function of estrogen and progesterone treatments. Everitt and his colleagues found estrogen treatment decreased NE turnover in the brainstem and cortex and the addition of progesterone enhanced this drop in turnover (Everitt et al., 1975). An earlier study demonstrated a drop in tyrosine hydroxylase activity 5-6 hours after progesterone was given (Beattie, Rogers & Soyka, 1972). So there is some evidence that the ovarian hormones could act to shut off noradrenergic activity. While norepinephrine turnover increases just before estrus (Wise, Rance & Barraclough, 1981), no one is sure of preoptic norepinephrine turnover

levels during the time of receptivity. Does NE turnover remain high during behavioral estrus and fall only as animals come out of heat, or are stores of norepinephrine used up to release GnRH to have synaptic norepinephrine quickly fall off to low estrus levels?

Manipulations of noradrenergic activity to alter receptivity levels have offered few definitive conclusions as to NE's role in the lordosis response. One of the earliest such manipulations was in a study by Bengt Meyerson. He found that the catecholamine-precursor L-DOPA, when given systemically would decrease lordosis levels and that blocking conversion of dopamine to norepinephrine reversed this effect (1964). A later study of his used amphetamines, which release catecholamines, and also found reduced receptivity levels (Meyerson, 1968; Eliasson, Michanek & Meyerson, 1972). One possible conclusion from these data would be that norepinephrine activity inhibits lordosis. However, amphetamines also release 5-hydroxytryptamine (5-HT, Costa, 1973) and Meyerson was more concerned with 5-HT's inhibitory role. Meyerson later found that the monoamine oxidase inhibitor pargyline also decreased lordosis frequencies, while L-DOPA added to this paradigm further decreased lordosis levels (Meyerson et al., 1973). Again Meyerson was more concerned with the increase in 5-HT levels than the increase in NE levels with pargyline. Ahlenius and his colleagues looked at the effect of diminution of noradrenergic activity, instead of its augmentation, on receptivity. In one study he injected tetrabenazine, which reduces catecholamine and 5-HT levels, and saw increases in lordosis levels (Ahlenius et al., 1972b). Blocking the enzyme tyrosine hydroxylase with

alpha-methyl para-tyrosine (*a*-MPT) also increased lordosis responding (Ahlenius et al., 1972a). They found that if 5-HT levels remained low while catecholamine levels returned to normal that lordosis behavior did not increase, arguing against a facilitative effect of the catecholamines. Another study confirmed that *a*-MPT would facilitate lordosis responding in rats and also found that the alpha-noradrenergic antagonists yohimbine and piperoxane had the same effect of increasing lordosis frequency, duration and intensity in estrogen-treated rats (Everitt et al., 1975). Meyerson repeated the finding that amphetamines, which increase NE levels, decreased lordosis (Michanek & Meyerson, 1977). These findings were duplicated by another group (Caggiula et al., 1979) which also confirmed that *a*-MPT could enhance receptivity (Herndon et al., 1978). This group added to their techniques the selective destruction of catecholaminergic neurons with 6-hydroxydopamine (6-OHDA), which they found would increase lordosis and amplify the facilitative effects of *a*-MPT (Herndon et al., 1978). However, Herndon found the opposite effect when he destroyed noradrenergic cell body nuclei with electrolytic lesions (Herndon, 1976). It is difficult to determine why this lesion has the opposite effect of Herndon's later use of 6-OHDA lesions. There is some suggestion that these lesioned animals were akinetic, lethargic or debilitated. Interpretation of these lesion studies is complicated because such gross manipulations affect a number of brain areas. Animals also tend to compensate for such lesions by receptor supersensitivity and other mechanisms (U'Prichard et al., 1979) so that a decrease in neurotransmitter content may not reflect a loss of that

transmitters' activity. With the exception of the last lesion study, reductions of noradrenergic activity by destroying norepinephrine neurons, disruption of norepinephrine synthesis or blockade of alpha-noradrenergic receptors facilitated lordosis behavior. Whereas, increasing noradrenergic activity by increasing its synthesis rate, increasing its release or decreasing its breakdown resulted in lower lordosis frequencies.

A complicating factor in these studies was the possibility that manipulations of hypothalamic norepinephrine levels would alter ACTH secretion from the pituitary and induce release of adrenal steroids. Such steroids might increase the incidence of lordosis. Early in the study of norepinephrine's effects on lordosis several groups found that adrenalectomies would eliminate the increases seen in lordosis responding resulting from injections of monoamine oxidase inhibitors (Feder & Ruf, 1969; Sodersten & Ahlenius, 1972; Eriksson & Sodersten, 1973). It was postulated that any facilitative effects of noradrenergic inhibitors was due to increased adrenal progestins in the blood. This may explain the long-latency increases in lordosis following monoamine. oxidase inhibition, but does not explain inhibition of lordosis in amphetamine and L-DOPA treated rats. Eriksson and Sodersten also found that adrenalectomy makes an animal more sensitive to the lordosis-enhancing effects of estrogen (1973). This may indicate that adrenalectomy greatly alters many physiological and neurological parameters. Conclusions derived from such preparations should be cautiously considered.

Use of systemic treatments to alter noradrenergic function has many drawbacks. Not only are large areas of the brain affected by such treatments but generally effects are not seen for several hours or even days, which increases the possibility that secondary effects, such as adrenal progestins, mediate the observed changes in behavior. A more immediate effect of systemic treatment was seen in rats treated with the alpha$_2$-noradrenergic agonist clonidine which reduced lordosis responding within thirty minutes (Davis & Kohl, 1977). This inhibitory effect of clonidine was blocked by pretreatment with the alpha-antagonist yohimbine. They interpreted these findings to indicate an inhibitory role of alpha-noradrenergic stimulation.

In apparent contradiction of an inhibitory effect of alpha-stimulation in the rat are data collected in the guinea pig. In guinea pigs the reduction of NE activity with the DBH inhibitor U-14624 decreases the percent of guinea pigs in heat (Nock & Feder, 1979). Giving the alpha-agonist clonidine 5 hours after U-14624 resulted in an increase in the percent of animals in heat but a decrease in the lordosis duration 1.67 hours later (Nock & Feder, 1979). Other studies from the same laboratory confirmed a facilitative role of norepinephrine on guinea pig lordosis (Crowley, Feder & Morin, 1976). Curiously, amphetamines did not facilitate receptivity in guinea pigs (Davis & English, 1977). It should be pointed out that guinea pigs are very different from rats in their receptivity patterns. Unlike rats, the occurrence of vaginal stimulation in guinea pigs will decrease the probability of lordosis on subsequent matings (Goldfoot & Goy, 1970).

Also, guinea pigs hold their lordosis much longer than rats (Dempsey, Hertz & Young, 1936). The finding that clonidine decreased lordosis duration may be important in this regard. It is possible that while noradrenergic activity shortens duration of lordosis in guinea pigs with long lordosis responses, it decreases lordosis frequency in rats with shorter responses. In any case, it is difficult enough to extrapolate the effects of noradrenergic manipulations across laboratories much less across species.

It was mentioned that systemic treatments affecting norepinephrine activity have the disadvantage of not being localizable and taking a long time to affect behavior. Direct placement of the neuroactive agent into the pertinent brain area ought to alleviate the former problem to the extent that the agent does not diffuse and may alleviate the latter problem. Only two studies have used this technique to look at noradrenergic activity and lordosis behavior. Ward and her colleagues implanted 9 - 11 ug of 5-HT blockers cinanserin and methysergide, the beta-blocker LB-46 and alpha-noradrenergic antagonist phentolamine into the medial preoptic-anterior hypothalamus, posterior hypothalamus or medial forebrain (Ward et al., 1975). They found that cinanserin, methysergide and LB-46 all increased lordosis quotients in estrogen-treated ovariectomized rats within 30 minutes when infused into the MPOA/AH. This increase was also seen when progesterone crystals were similarly implanted. The other study infused solutions of noradrenergic agents into the MPOA or the ventromedial hypothalamus (Foreman & Moss, 1975). These researchers showed that with repeated

matings infusions of norepinephrine and beta-noradrenergic agonists would increase lordosis responding to a peak at 105 minutes after infusion. When, however, the 105 minute test was the first post-infusion test, NE no longer increased lordosis/mount ratios (in fact, there was a slight decrease). Also the alpha-noradrenergic agonist methoxamine significantly inhibited lordosis. The fact that NE could increase the facilitative effect of repeated matings (Rodriguez-Sierra, Crowley & Komisaruk, 1975; Rodriguez-Sierra, Crowley & Komisaruk, 1977) but was unable to increase lordosis frequencies when the animals were given only one test may reflect norepinephrine's analgesic effect with respect to vaginal stimulation (Komisaruk, 1982). There has yet to be a well-controlled study which looks at the more immediate effects of preoptic NE infusions on lordosis frequencies. Such a study should infuse a range of NE doses into various brain areas, including the important MPOA, and look at its effects on lordosis after a very short time before the exogenous NE is degraded by endogenous enzymes and before secondary effects of such a manipulation can occur. The following experiments are designed to address these questions using such techniques.

GENERAL METHODS

Animals

Sherman strain female rats used in these experiments were obtained from a commercial supplier (Camm, Wayne, N.J.) when they were 60-90 days of age. After implantation of bilateral intracerebral cannulae, females were caged singly and maintained on a reversed dark-light cycle with a 10 hour dark phase beginning at 1100 hours. All rats were provided food and water ad libitum.

Surgery

All animals were bilaterally ovariectomized under ketamine (Vetalar, Parke-Davis, Detroit) anesthesia 3-5 days after arrival in the laboratory.

No less than one day after a screening test (see below) animals were bilaterally implanted under ketamine anesthesia. Implant cannulae were of 23 gauge stainless steel tubing (Small Parts Inc., Miami, Fla.). Two of these cannulae were preset 1.6-1.8 mm apart with Kadon dental acrylic. This cannula assembly was lowered to 1 mm above the target site. Medial preoptic area sites were determined to be directly under the bregma, equidistant on either side of the midline, and 3.4 - 4.0 mm

above vertical zero (Konig & Klippel, 1963). The two 23 gauge cannulae were then fitted with 27 gauge stainless steel inserts, measured to extend 1 mm beyond the outer cannulae and into the target site. In order to secure the cannula assembly to the skull four set screws were drilled into the skull and Kadon dental acrylic applied around the screws and assembly base.

Testing Procedures

In all sexual behavior tests females were permitted ten mounts by sexually experienced Long-Evans males. Males were allowed to adapt to the testing arena (45 X 50 X 58 cms. Plexiglas; floored with 4 - 7 cms. of Sanicel) for several minutes before the introduction of a female. If a male failed to mount a female 10 times the female was introduced to another male in a different testing arena. A mount was counted when the male palpated the female's flank and exhibited pelvic thrusting. During a mount the female was recorded as having shown or not shown lordosis behavior. Lordosis behavior was then measured as a lordosis quotient (LQ) which is defined as the frequency of lordosis responses to ten mounts divided by ten mounts and multiplied by 100. Any animals not achieving a screening test LQ of 70 or higher was retested. If the animal again failed to respond it was eliminated from the experiment. All females were injected with a dose of estradiol benzoate (EB) 72, 48, and 24 hours before testing. Some animals were also injected with progesterone 4 - 5 hours before testing. All hormones were dissolved in sesame oil.

Drugs and Infusion

Noradrenergic agents used in these experiments include the agonists: norepinephrine (-Arterenol, Sigma, St. Louis,MO). - epinephrine (Sigma, St. Louis, MO), clonidine (Catapres @, Boehringer-Ingelheim, Ridgefield, CO), methoxamine Burroughs- Wellcome Co., Triangle Park, NC), phenylephrine (Sigma), and isoproterenol (Sigma). There were also three noradrenergic antagonists yohimbine (Sigma), phentolamine (CIBA-Geigy, Summit,NJ), and propranolol (Sigma). As a neuractive control the putative neuro transmitter 5-hydroxytryptamine (Sigma) was also infused.

After determining their pre-infusion receptivity levels all animals achieving test criteria (LQ not more than thirty for facilitation tests and not less than seventy for inhibition tests) were then infused by a microinfusion pump (Harvard Apparatus, Special; Millis, MA.). Inserts were first removed to provide an open space in the target site for infusates. Doses of each agent were premeasured and stored in parafilm covered 12 X 75 mm culture tubes at appropriate storage temperatures. Immediately before infusions artificial cerebrospinal fluid (CSF; containing: 130 mM NaCl,25 mM $NaHCO_3$, 5 mM $NaPO_4$, 30 mM KCl, 8 mM $MgCl_2$, and 13 mM $CaCl_2$; pH=6.8) or isotonic saline (0.9%) was added with a 1000 ul micropipette to the appropriate culture tube. This infusion fluid or infusate was then drawn into an infusion cannula (28 gauge) which was attached to a vehicle-filled syringe by polyethylene tubing (20 gauge). The infusion insert was measured to extend to 1 mm beyond the end of the cannula guide. The animals were then infused for 30 seconds per cannula

at 1 ul/minute during which time the animals were allowed freedom of movement. After infusion all inserts were replaced.

Histology

Following completion of all tests animals were perfused with intracardial injections of 9% saline followed by 10% phosphate-buffered formalin under pentobarbital anesthesia. Brains were then blocked and set in 10% gelatin. These blocks were then frozen and sectioned coronally to a 50 um thickness. Representative sections were stained with neutral red stain. Implant sites were determined by an independant observer or by the author without access to an animal's behavioral performance. Data from animals that did not have implants in the medial preoptic area were eliminated from statistical analysis for that area.

All norepinephrine implant sites were separately analyzed as facilitative responders if the animal with that implant showed an increase in LQ of 50 or more over pre-infusion levels. Animals were evaluated as inhibitory reponders if the animal demonstrated a drop in LQ of 40 or more from pre-infusion test levels. These implant sites were then compared using Fisher's exact probability tests between MPOA and other areas (areas bordering the MPOA such as the anterior hypothalamus and lateral preoptic areas were eliminated from this statistical comparison).

Experiment 1

It seems reasonable that norepinephrine plays a role in the control of pituitary hormones and thus female fertility (see Background). Norepinephrine's part in the control of female receptivity is much less certain. Foreman and Moss (1978) infused noradrenegic substances and saw increases in lordosis over an hour and a half later and claimed that norepinephrine (NE) facilitates lordosis. Whereas other researchers saw increases in lordosis performance after systemic treatments which reduce norepinephrine activity (Ahlenius et al., 1972a; Ahlenius et al., 1972b; Everitt et al., 1975; Ward et al., 1975; Herndon et al., 1978; Caggiula et al., 1979) and reductions in responding following increases in noradrenergic activity (Meyerson, 1964 & 1968; Michanek & Meyerson 1977; Eliasson, Michanek & Meyerson, 1972; Caggiula et al., 1979) and after injection of the alpha-noradrenergic agonist clonidine (Davis & Kohl, 1977). Thus systemic treatments which enhanced norepinephrine activity reduced lordosis behavior whereas those which destroyed norepinephrine neurons or temporarily impaired their release of NE increased receptivity. The systemic treatments took a long time to have their inhibitory effects. The first experiment was to infuse NE directly into the MPOA to see if a short-latency inhibitory effect could be evidenced.

Methods

Preliminary experiments in this laboratory indicated that infusions of 10 and 20 ug doses of NE into the MPOA would reduce receptivity in estrogen-progesterone treated female rats. To elicit high levels of receptivity all animals were injected with 0.5 ug estradiol benzoate (EB) for three days and 500 ug progesterone on the fourth day 4-5 hours before the first test. Twelve animals were randomly assigned to a three dose series (one treatment/week) in a Latin Square design. The three doses were 10 ug/animal and 20 ug/animal of NE and a CSF control dose. Animals were tested before and at 15, 45 and 90 minutes after infusion. Animals not attaining a pre-infusion LQ of 70 or above were eliminated from statistical analysis. As in all of the following experiments, at least two different doses were infused every day in order not to confound treatment effects with effects of the testing days.

Statistical comparisons were done on log-transformed data. Analysis consisted of a four-factor analysis of variance (time x dose x week x animal). Further analysis was done with Dunnett's t-tests.

As a control infusate the reportedly inhibitory neurotransmitter serotonin or 5-hydroxytryptamine (5-HT, see Meyerson & Lewander, 1970; Everitt, Fuxe & Jonsson, 1975) was infused into the MPOA. Here seven animals were randomly assigned to a three dose series as above. This design used 1 and 2 ug/animal doses of 5-HT and CSF as a control vehicle. Five other animals were treated with 20 ug of 5-HT. These animals were hormonally treated as above. All other test parameters

were also duplicated.

Results

Both doses of NE significantly inhibited lordosis behavior 15 minutes after infusion (CSF versus 10 ug, Dunnett's $t=6.36$, $d.f.=2,14$ $p<.01$; CSF versus 20 ug Dunnett's $t=5.33$, $d.f.=2,11$ $p<.01$). The inhibitory effect of norepinephrine was only transient as lordosis responding returned to pre-infusion levels for the 45 and 90 minute tests (see Figure 1). Because this effect was transient and only appeared during the first post-infusion test there was no significant effect of NE dose across all three post-infusion tests ($F= 5.34$, $d.f.=2,4$ $p>.10$). There were significant effects across test times and weeks tested (see Appendix A). This short-latency reduction in lordosis frequency is not attributable to animal debilitation. No animals exhibited any locomotor dysfunction. Only a few animals were slightly slowed after NE treatment.

The control infusions of 5-HT did not significantly reduce lordosis behavior at any dose (see Figure 2). None of these animals were debilitated or obviously behaviorally affected by the serotonin infusions.

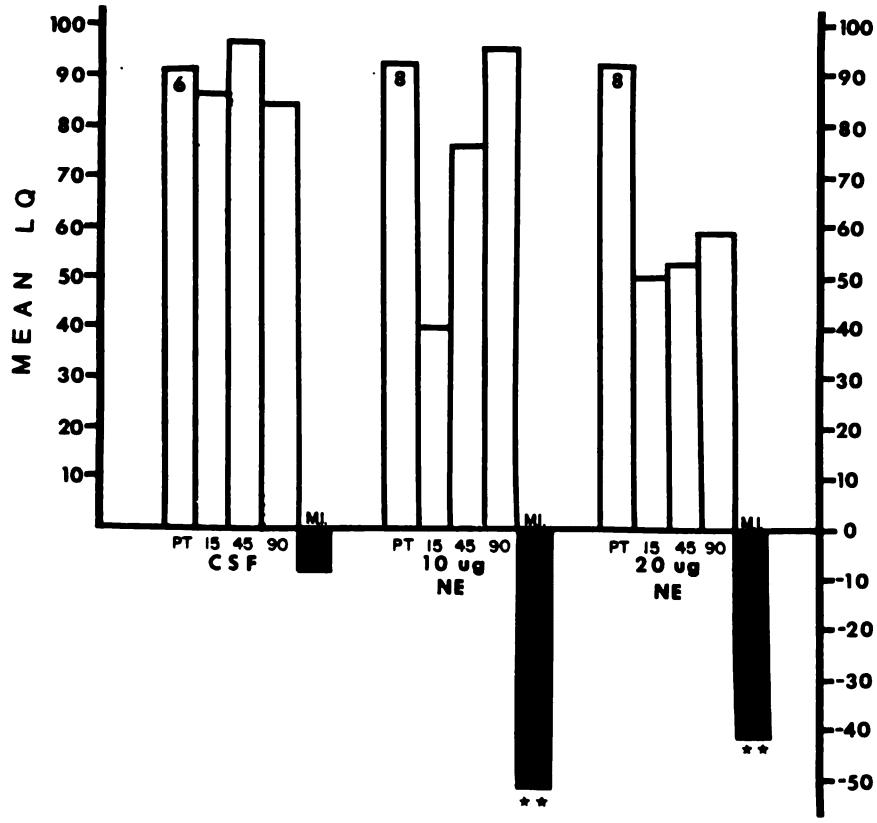

FIGURE 1

Infusion of 10 or 20 µg doses of norepinephrine (NE) significantly inhibited lordosis in estrogen-progesterone treated ovariectomized rats. Numbers in the pre-infusion test (PT) bars indicate the number of animals per group. Maximal inhibition (M.I. = lowest post-infusion LQ minus pre-infusion LQ) was seen at 15 minutes after NE infusion at both doses. This inhibition, seen at both doses, was transient and significant (** = $p < .01$ for Dunnett's t-test, see Appendix A). Each animal was infused with artifical cerebrospinal fluid (CSF) alone or with 10 or 20 µg NE in a one µl volume over one minute. These three treatments were distributed to each animal across the three weeks of testing in a Latin Square design.

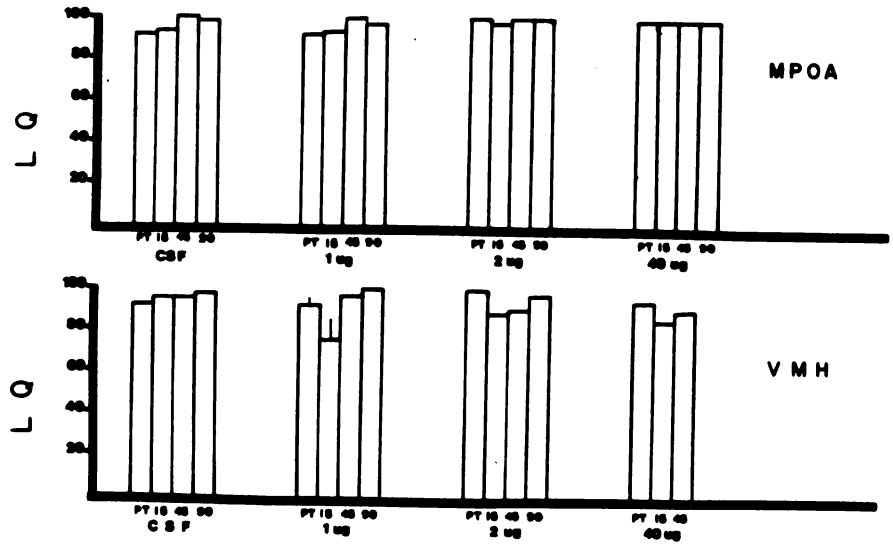

FIGURE 2

Serotonin infusions into the medial preoptic area (MPOA) or ventromedial hypothalamus (VMH) did not affect receptivity. Seven MPOA implanted and six VMH implanted animals were infused with CSF, 1 or 2 µg serotonin (5-HT). Each animal received a treatment series counterbalanced across the three weeks of once a week testing. Five other MPOA and four VMH animals were infused with 40 µg/animal 5-HT. Animals were made receptive with estrogen and progesterone injections (0.5 µg EB for three days followed by 500 µg progesterone). No test found any significant effect of 5-HT infusions.

Summary

The 10 and 20 ug doses of norepinephrine significantly reduced lordosis behavior at 15 minutes. This inhibitory effect did not continue at 45 minutes after infusion. The putative neurotransmitter serotonin did not show such an inhibitory effect when infused into the MPOA.

Experiment 2

One observation that could be derived from experiment 1 was that the 10 and 20 ug doses of NE were capable of inhibiting lordosis behavior 15 minutes after infusion. A second observation was that lordosis quotients had recovered to pre-infusion levels after 45 minutes. Although animals did not demonstrate any behavioral debilitation the possibility remained that 10 and 20 ug doses had pharmacological rather than physiological effects. In an attempt to avoid this four lower doses of NE (0.5, 1, 2 and 5 ug) were infused along with the 10 ug dose. Because the 15 minute observation was the only post-infusion test where a significant drop in receptivity was seen, the possibility existed that the point of maximal inhibition occurred at some time earlier than 15 minutes after infusion. In order to test this possibility animals were first tested 3-5 minutes after infusion and then again at 20 minutes.

Norepinephrine can act at different types of receptors (see Background). If the inhibitory effect of norepinephrine is exclusive to one of these receptors then it ought to be possible to block the effect with the appropriate noradrenergic receptor antagonist. Different noradrenergic antagonists were given simultaneously with NE to determine which receptors NE might be acting on to inhibit lordosis. These antagonists have been shown by radioligand assay to bind with high affinity to specific types of noradrenergic receptors (Greenberg, U'Prichard & Snyder, 1976; Berthelsen & Pettinger, 1977; U'Prichard et

al., 1979). The antagonists used included phentolamine, a reputed $\alpha_1$antagonist, yohimbine an alpha$_2$blocker, and propranolol which shows higher affinities to beta-receptors than alpha-receptors (Berthelsen & Pettinger, 1977; Franklin & Herberg, 1977; U'Prichard & Snyder, 1979; and see Background).

Methods

Eighteen females were randomly assigned to three groups. Each group was to be infused with a different dose of NE (2,5 and 10 ug/animal). Within each group each animal was assigned to a three-treatment series (treatment/week) as seen in experiment 1. These three treatments consisted of an infusate solution of 5 ug/ul yohimbine, 5 ug/ul phentolamine, or saline vehicle added to the NE dose just prior to infusion. Saline was used as a vehicle because of difficulties dissolving yohimbine in the artificial CSF solution. This design was repeated for three lower doses (0.5, 1 and 2 ug/animal) of NE using eighteen other animals. Eight different animals were treated using a simple two-week crossover design with animals receiving 2 ug NE with either artificial CSF or 5 ug/ul propranolol. All animals were hormonally treated as in experiment 1. Receptivity tests were conducted before infusion and 5 and 20 minutes after infusion. Inhibition was measured as a change in lordosis quotient between the pre-infusion and the 5 minute test. Paired t-tests were done on these differences for each group. Kruskal-Wallis tests compared both across the various NE doses and compared NE treatment to NE plus antagonists at all doses.

Further comparisons were done, where appropriate with a non-parametric Dunn's test (Hollander & Wolf, 1972) and Mann-Whitney U tests (Siegel, 1956).

Results

Norepinephrine reduced lordosis responding five minutes after its infusion at all five doses (see Figure 3). This reduction was significant at the 2, 5 and 10 ug NE dosages. It might be noted that for all NE doses lordosis responding returned to control levels by the 20 minute test. Only at the 10 ug dose does lordosis remain depressed at the 20 minute test. It is also only at this highest dose that any lethargy was seen after infusion. The two lowest doses (0.5 and 1 ug) did not significantly attenuate receptivity. At the 5 and 10 ug doses none of the noradrenergic antagonists blocked the inhibition of lordosis (see Table 1). For the 5 and 10 ug doses all infusate combinations showed significant inhibition of lordosis at 5 minutes. At 2 ug NE, the lowest effective ihibitory dose, the alpha-2 antaonist yohimbine appears to block the reduction in receptivity effected by norepinephrine (see Figure 4). As indicated in Figure 4, at the 2 ug dose the addition of 5 ug/ul propranolol or phentolamine had no effect on the norepinephrine induced drop in lordosis responding. There is no significant reduction in lordosis at 5 minutes if yohimbine is added to the infusate. Thus only the alpha-2 antagonist yohimbine showed any capacity to block the significant reductions in lordosis seen at 5 minutes after infusion.

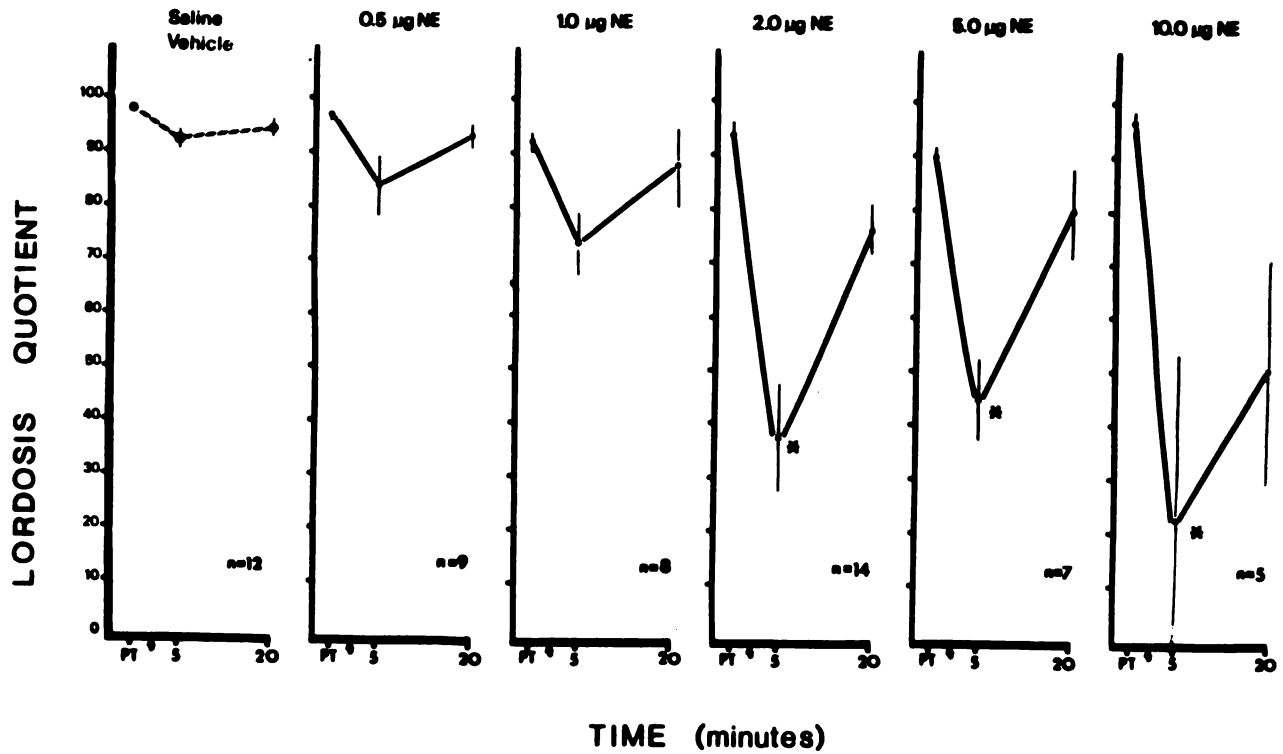

FIGURE 3

Norepinephrine infusions into the MPOA resulted in a transient inhibition of lordosis behavior. Fifty-five ovariectomized rats treated with estrogen (0.5 µg for 3 days) and progesterone (500 µg on the fourth day 4-5 hours before testing) showed high pre-infusion test (PT) lordosis quotients. Infusions of 2, 5 and 10 µg NE doses significantly reduced lordosis quotients at a 5 minute post-infusion test (comparing the reduction in LQ from PT to 5 minutes in NE-infused animals versus saline vehicle-infused animals, * = $p < .05$ for both Mann-Whitney and Dunn's nonparametric tests). Doses of 0.5 and 1 µg NE did not significantly reduce lordosis quotients. All infusions (marked with arrows) were made in saline vehicle.

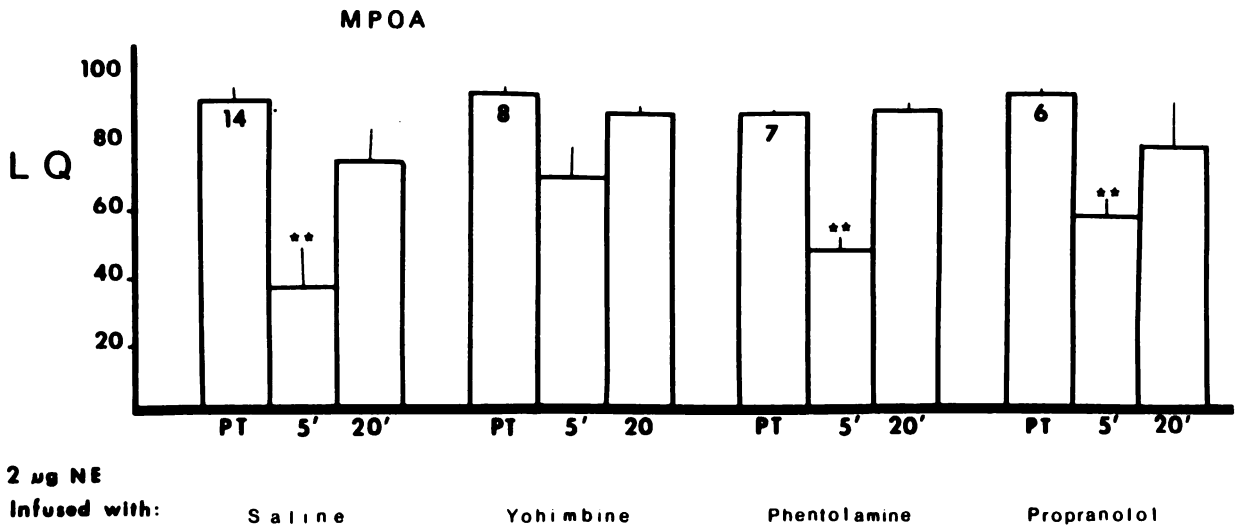

FIGURE 4

Yohimbine was the only noradrenergic antagonist used that was able to block the inhibitory effect of norepinephrine. All antagonists were infused at a concentration of 5 µg/µl. All antagonists were infused simultaneously with 2 µg NE into the MPOA. Numbers in pre-infusion test (PT) bars indicate number of animals per group. Neither the beta-noradrenergic antagonist propranolol nor the alpha-antagonist phentolamine were able to block the significant decrease in lordosis after NE infusion (** = $p < .01$ on paired t-test of pre-infusion LQ versus 5 minute test; see Appendix B).

TABLE 1

Mean lordosis quotients (± SE) of estrogen-progesterone treated ovariectomized rats before and after infusion of norepinephrine simultaneoulsy with alpha-antagonists.

Norepinephrine Dose	Infusate	N=	Preinfusion Test	5 Minute Test	20 Minute Test
0.5 µg	Saline	9	9.77 ± .04	8.44 ± .52	9.33 ± .19
0.5 µg	Yohimbine	6	9.83 ± .02	7.83 ± .8	8.16 ± .18
0.5 µg	Phentolamine	5	9.8 ± .02	9.16 ± .3	9.8 ± .02
1 µg	Saline	8	9.25 ± 7.37	7.37 ± .59	8.86 ± .64
1 µg	Yohimbine	7	9.14 ± .26	5.57 ± 1.9 ϕ	7.28 ± 1.49
1 µg	Phentolamine	7	9.43 ± .07	6.81 ± 1.6	9.71 ± .07
2 µg	Saline	14	9.35 ± .06	3.85 ± 1.0 Ψ $\phi\phi$	7.64 ± .51
2 µg	Yohimbine	8	9.37 ± .09	7.0 ± .87	8.87 ± .14
2 µg	Phentolamine	7	8.71 ± .19	4.86 ± .22 $\phi\phi$	8.85 ± .26
5 µg	Saline	7	9.0 ± .2	4.43 ± .81 Ψ $\phi\phi$	8.0 ± .77
5 µg	Yohimbine	6	9.66 ± .03	5.33 ± 1.5 ϕ	7.6 ± 1.4
5 µg	Phentolamine	6	9.66 ± .09	3.16 ± 2.1 Ψ $\phi\phi$	8.83 ± .3
10 µg	Saline	5	9.58 ± .05	2.2 ± 3.0 Ψ ϕ	5.06 ± 2.3
10 µg	Yohimbine	5	9.4 ± .13	3.2 ± 1.1 $\Psi\Psi$ $\phi\phi$	3.6 ± 2.13
10 µg	Phentolamine	5	9.0 ± .32	3.8 ± 2.67 $\phi\phi$	5.8 ± 2.4

Mann-Whitney U test ϕ = $p < .05$; $\phi\phi$ = $p < .01$

Dunn's non-parametric test Ψ = $p < .05$; $\Psi\Psi$ = $p < .01$

The Kruskal-Wallis test demonstrated a significant effect of NE dose on the drop in LQ seen at 5 minutes ($H' = 12.28$, $p<.05$). Use of the Mann- Whitney U tests to compare NE induced inhibition versus vehicle-infused drops found 2,5 and 10 ug dosages to significantly reduce LQs (see Appendix B). Using the Dunn's test only the 2 and 10 ug doses showed significant differences from saline-infused controls (2 ug vs. saline = 19.88, $p<.01$; 10 ug vs. saline = 22.94, $p<.01$). Paired t-tests of pre-infusion test LQs versus the 5 minute test LQs are summarized in Appendix B. This statistical analysis confirms the inhibitory effect of the 2, 5 and 10 ug doses. Paired t-tests also show no significant drop in LQs with 5 ug/ul yohimbine added to the 2 ug dose of NE. No Kruskal-Wallis test showed a significant difference among antagonist treatment groups at any NE dose (H' for 0.5 ug = 4.11; for 1 ug=4.88; for 2 ug=4.67; for 5 ug=3.36; for 10 ug=0.735). However, use of Dunn's test to probe the 2 ug NE group found that the addition of either phentolamine or propranolol to 2 ug NE fails to block its inhibitory effect (saline vehicle alone versus 2 ug NE with phentolamine Dunn's $q=14.7$, $p<.01$; versus 2 ug NE with propranolol $t= 13.5$, $p<.05$). But adding yohimbine to this lowest effective dose blocks the significant drop in lordosis frequency seen with NE (saline vehicle inhibition versus 2 ug NE with yohimbine $q=7.06$, $p>.05$).

Analysis of the implant sites of the animals in experiments 1 and 2 found evidence for localization of the NE inhibition to the MPOA. Animals with implants located in the MPOA showed inhibition more often than those with implants in other brain sites (Figure 5). Animals

infused into the medial basal hypothalamus showed no significant reductions in lordosis at either 2 or 5 ug NE doses (see Figure 6).

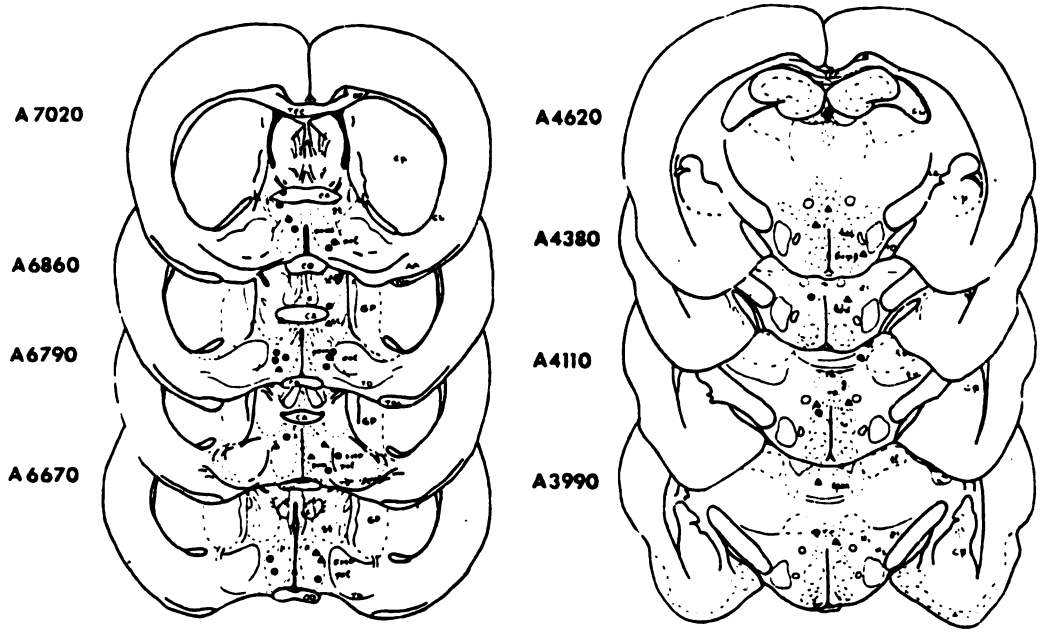

FIGURE 5

Analysis of these representative implant sites demonstrates that the inhibitory effect of NE infusion is localized in the medial preoptic area (pom above, coronal sections adapted from Konig & Klippel, 1963, by J. Kaminski). Receptive females from experiments 1 and 2 were used in this histological analysis. Animals in which NE infusions resulted in a reduction of lordosis quotient of 40 or more are designated as responders (solid circles). All others were non-responders (solid triangles).

Location of implant	# responders	# non-responders
MPOA sites	30 Ω	9 §
Non-MPOA sites	12	22

Ω Fisher's test $F=11.24$, $p<.001$

§ Chi-square versus responders for MPOA, $\chi^2=11.3$, $p<.001$

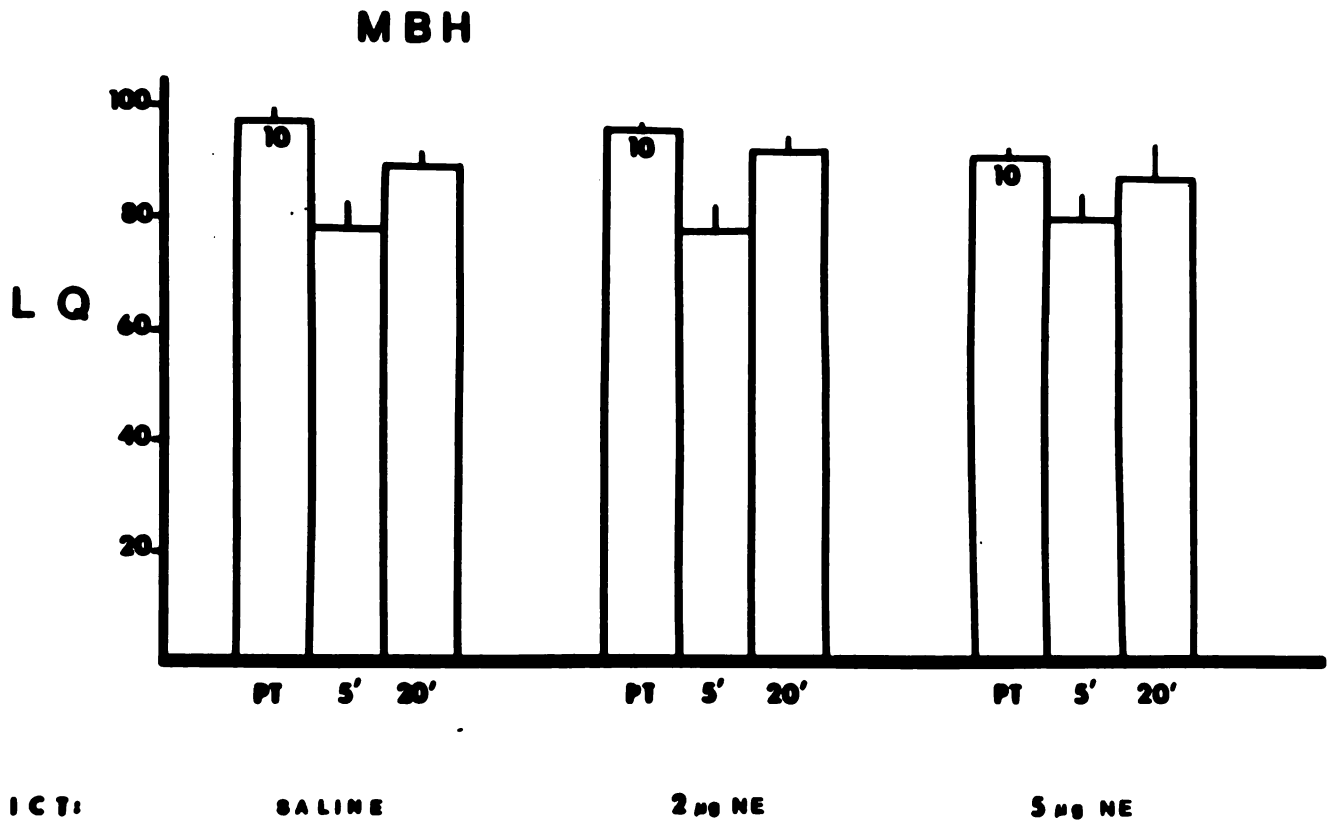

FIGURE 6

Infusion of NE into the medial basal hypothalamus (MBH) did not alter receptivity. Receptive estrogen-progesterone treated ovariectomized rats were bilaterally infused into the MBH with 2 or 5 µg NE or saline. Tests were conducted before (pre-infusion = PT) and 5 and 20 minutes after infusions. There was no significant inhibition of lordosis at either NE dose as was seen at the same doses of NE infused into the MPOA (see Figure 3).

Summary

Norepinephrine inhibited lordosis responding within 5 minutes at doses as low as 2 ug/animal. This experiment is the first to demonstrate an effect on receptivity with infusions of neuroactive drugs, with this short a latency. The antagonist data may indicate that yohimbine is capable of blocking this norepinephrine induced inhibition at this lowest effective dose. No such attenuation was seen with either phentolamine or propranolol. These findings may indicate that norepinephrine inhibits lordosis behavior by stimulating alpha$_2$ noradrenergic receptors.

Experiment 3

In experiment 2 norepinephrine induced a short-latency, transient reduction in lordosis frequency. The alpha-antagonist yohimbine was the only agent which demonstrated any capacity to block this effect of NE. However, the transient nature of norepinephrine's inhibitory effect on behavior made the discovery of an adequate antagonist dose and timing of antagonist administration somewhat difficult. It was decided instead to attempt to use a series of specific noradrenergic receptor agonists to replicate the inhibition seen with norepinephrine. Three agents with high affinity for alpha-receptors and one with greater affinity for beta-receptors were chosen for this purpose. The first two alpha-agonists, methoxamine and phenylephrine, have reportedly greater affinity for $alpha_1$-receptors (Berthelsen & Pettinger, 1977; U'Prichard & Snyder, 1979). The third agent, clonidine, is widely used as a potent agonist of $alpha_2$-type receptors (Greenberg, U'Prichard & Snyder, 1976; Berthelsen & Pettinger, 1977; U'Prichard & Snyder, 1979; Young & Kuhar, 1981; Aghajanian & VanderMaelen, 1982; Estes, Simpkins & Kalra, 1982; Leung et al., 1982). A standard agonist, isoproterenol, was used to test if the inhibitory effect of norepinephrine might not be mediated via the beta-receptors. The fourth agent is epinephrine, the metabolite of norepinephrine (see Background). Like norepinephrine epinephrine acts of both alpha- and beta-receptors. There is some evidence that epinephrine has a slightly greater affinity for $alpha_2$- than

alpha$_1$-receptors (U'Prichard, Greenberg & Snyder, 1976; Berthelsen & Pettinger, 1977; U'Prichard & Snyder, 1979).

Methods

Sixty animals were randomly assigned to agonist infusate groups. All animals were infused with CSF, and two doses of their agonist. (Due to difficulties dissolving epinephrine in CSF, 1 ml of .25 M ascorbic acid was added to the NE dose and then diluted with 3 ml CSF. The control CSF for this group also contained .25 M ascorbic acid.) Doses were 2 or 5 ug for all agonists except clonidine which, because of its potency, was infused at 0.5 and 1 ug doses. These three treatments were counterbalanced across the three weeks of testing. All animals were estrogen and progesterone treated to show receptivity as in experiments 1 and 2. Statistical analyses used paired t-tests of pre-infusion LQs minus the lowest post-infusion test LQs. Kruskal-Wallis tests were also used to compare test scores of CSF vehicle-infused animals with LQs for agonist-infused animals. Further testing was done with Dunn's non-parametric test and Mann-Whitney U comparisons.

Results

The two most potent inihibitory agents were clonidine and epinephrine (see Figures 7 & 8). These two agents also are the two out of the five with the greatest affinity for alpha$_2$-receptors. As seen in Table 2, the only two agonists to significantly inhibit lordosis responding were epinephrine and clonidine. Epinephrine infusions, at 2

and 5 ug, resulted in significant reductions in lordosis at 5 minutes. This reduction was transient because receptivity recovered to control levels by the 20 minute test. This effect replicates the results of equal doses of NE. The transient nature of this reduction is not surprising since it has been suggested that norepinephrine and epinephrine are neurotransmitters in the MPOA and that their catabolic enzymes ought to be in situ to deactivate them (see Background). Clonidine maintained its inhibitory effect at the 20 minute test (see Appendix C). Unlike epinephrine and norepinephrine, clonidine is probably not an endogenous brain substance and therefore would not be metabolized as quickly to inactive forms. The beta-adrenergic agonist isoproterenol resulted in no significant drop in lordosis responding. The two agonists which demonstrate a greater affinity for $alpha_1$-receptors, methoxamine and phenylephrine, did not demontrate a reduction in LQ as with norepinephrine, epinephrine or clonidine. Methoxamine infusions did not result in a significant reduction, whereas phenylephrine showed a significant reduction in LQ at the 2 and not the 5 ug dose using the Mann-Whitney U test (see Table 2 and Appendix B). None of these animals were debilitated nor were they lethargic.

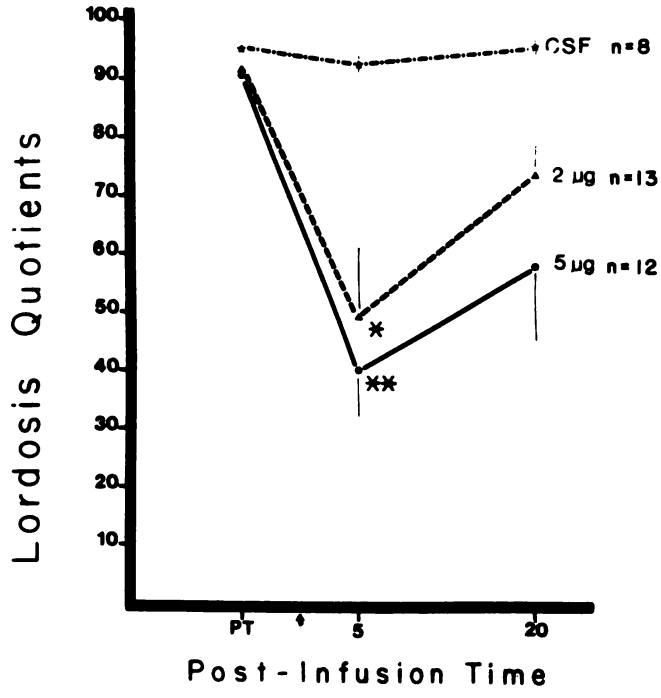

FIGURE 7

Epinephrine infused into the MPOA resulted in a transient suppression of receptivity within 5 minutes. Animals were made receptive with estrogen and progesterone injections. Three intracerebral treatments, CSF vehicle with .25 M ascorbic acid (see text) alone or with 2 or 5 µg/µl epinephrine, were counterbalanced across three weeks of testing. Infusions of 2 or 5 µg/animal of the alpha-agonist epinephrine resulted in significant reductions in lordosis behavior 5 minutes later (comparing epinephrine to control infused animals' LQs with Dunn's test * = $p < .05$, ** = $p < .01$, see also Table 2).

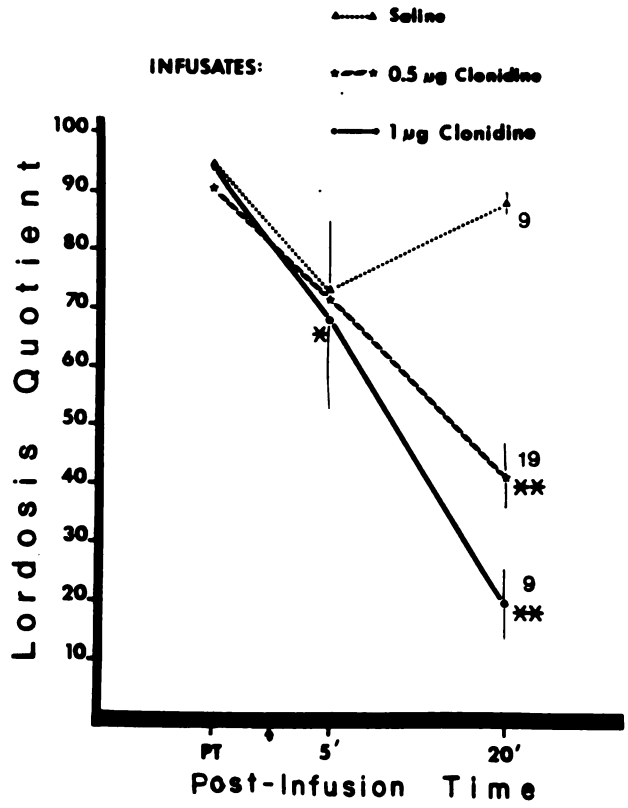

FIGURE 8

Clonidine infused into the MPOA resulted in a prolonged reduction in lordosis levels. Estrogen-progesterone treated receptive animals were infused with CSF vehicle alone or with 0.5 or 1 µl/animal of the alpha$_2$-agonist clonidine. These three treatments were counterbalanced across three weeks of once a week testing. Both doses of clonidine reduced lordosis frequencies at 5 and 20 minutes after infusion. This inhibition was significant at both doses at the 20 minute test (* = $p < .05$, ** = $p < .01$, for Mann-Whitney U tests comparing these doses to LQs for vehicle-infused animals).

TABLE 2

Mean lordosis quotients (± SE) of estrogen-progesterone treated ovariectomized rats before and after infusions of noradrenergic agents

Agent	Dose µg	N=	pre-infusion test	5 minute post-infusion	20 minute post-infusion
Norepinephrine	2	14	9.35±.06	3.85±1.0 ϕϕ Ψ	7.6±.51
	5	7	9.0±.2	4.43±.81 ϕϕ	8.0±.77
Epinephrine	2	13	9.14±.06	4.83±1.1 ϕ Ψ	7.4±.51
	5	12	9.16±.06	4.0±.79 ϕ ΨΨ	5.75±1.3
Clonidine	0.5	19	9.25±.04	7.21±.39	3.8±.56 ϕϕ ΨΨ
	1	9	9.44±.1	6.6±1.4 ϕ	2.0±.54 ϕϕ ΨΨ
Phenylephrine	2	9	9.66±.05	6.77±1.0 ϕ	8.55±.35
	5	11	9.06±.09	7.9±.88	9.35±.12
Methoxamine	2	6	9.16±.08	7.5±.71	9.5±.04
	5	5	9.2±.11	7.2±2.7	8.6±1.1
Isoproterenol	2	9	9.66±.05	7.74±.64	8.54±.1
	5	8	9.62±.06	8.72±.24	8.72±0.25

Mann-Whitney U test ϕ = $p < .05$; ϕϕ = $p < .01$

Dunn's non-parametric test Ψ = $p < .05$; ΨΨ = $p < .01$

Summary

Clonidine inhibited lordosis at 0.5 and 1 ug/animal doses for up to twenty minutes. Epinephrine demonstrated a more transient inhibition of lordosis at 2 and 5 ug doses in the MPOA. The epinephrine-induced inhibition closely replicated that of norepinephrine infusions at the same doses. The same doses of the $alpha_1$-agonists methoxamine and phenylephrine as well as the beta-adrenergic agonist isoproterenol failed to reduce receptive behavior. A scale of lordosis inhibitory effectiveness would be, from most inhibitory to least: clonidine > epinephrine = norepinephrine > methoxamine = phenylephrine = isoproterenol. This order would closely represent these substances' affinity to $alpha_2$-receptors as seen in radioligand assays (Greenberg, U'Prichard & Snyder, 1976; Berthelsen & Pettinger, 1977; U'Prichard & Snyder, 1979).

Experiment 4

This experiment was conducted to determine if norepinephrine had any facilitative effect on lordosis behavior. Although the bulk of evidence involving systemic treatments showed that blockage of noradrenergic neural transmission increased female receptivity in rats (see Background), the only other study involving intracerebral infusions of NE or noradrenergic agonists claimed an increase in lordosis/mount ratios after 105 minutes (Foreman & Moss, 1978). The following experiment was undertaken in an effort to replicate their findings and determine a dose-response relationship for this supposed facilitative effect. There was some concern that an effect on behavior of over an hour and a half latency may be due to alteration of adrenal progestin output. Therefore, an attempt was made to control for this possibility by providing the animals with adequate progesterone levels and to limit receptivity by reducing estrogen levels.

Method

Experiment 4 consisted of three separate experimental designs. In the first design sixty-three animals were injected with estrogen (0.5 ug EB) for three days prior to testing and received no progesterone. This hormone treatment is insufficient to elicit receptivity. Animals were tested before and 15, 45 and 90 minutes after infusions. To avoid the

complications of prolonged implants animals were tested for two weeks after implantation. Each animal was then bilaterally infused with two of the six doses (0.1, 0.3, 0.62, 1.25, 2.5 & 10 ug) of norepinephrine given. Animals were randomly assigned to these incomplete blocks in whole plots. A split-plot repeat-measure four-way analysis of variance (dose x time x weeks x animals), commensurate with this design was used for statistical analysis (Gill, 1970).

In the second design, eight other animals were given the same hormonal treatment. In a two-week crossover design animals were infused with CSF one week and 20 ug NE the other. A three-way ANOVA was used to analyze the initial data. Scheffe's interval tests were done to compare CSF to 20 ug NE for both weeks. Further analysis comparing CSF with all NE doses was done using Dunnett's t-tests.

In the third design animals were injected with 0.175 ug EB for three days followed by 500 ug progesterone on the fourth day 4-5 hours before testing. Eighteen animals were tested for three weeks receiving infusions of one dose of NE (2.5 or 10 ug) or CSF each week. A latin square design was used to counterbalance treatments across the three weeks. These data were analyzed with a four-factor analysis of variance (time x dose x week x animal). In all these designs only animals with implants in the MPOA and showing pre-infusion LQ scores of 30 or below were used in the statistical analysis. Further tests were done with Dunnett's t-tests which compared CSF controls with the two NE doses. The 90 minute test scores for these animals were compared to those for the same doses in pre-infusion LQ scores of 30 or below were used in the

statistical analysis.

Results

There is little evidence for a direct facilitative effect of norepinephrine infusions on lordosis. Infusions of six doses of norepinephrine did not demonstrate a significant dose-response relationship (see Figure 9). There was a general increase in lordosis across time after infusion (see Appendix C; observation time $F=3.99$, $d.f.=2,159$; $p<.05$) which was also seen in animals treated only with CSF ($F=9.7$, $d.f.=3,18$, $p<.01$). This overall increase may have masked any effect across doses. Therefore, lordosis quotients for the highest mean post-infusion test, the 90 minute test, were compared with t-tests versus that for CSF treated animals. There were no significant differences in lordosis response levels between NE infused animals and vehicle infused controls (see Appendix C). Of the few animals that did have increased LQs after NE infusion, no more had implants in the MPOA than in other brain sites (Figure 10). The only NE dose which was followed by significant increases in lordosis, was the 20 ug dosage (see Figure 11; Scheffe's interval test for 15 minute test $Q=3.65$, $d.f.=1$, $p<.01$; for 45 and 90 minute tests $Q=2.25$, $d.f.=1$, $p<.05$ for both times). However, many of the animals receiving this dose were lethargic after infusion. This suggests the possibility that lordosis responding increased because lethargic females were more accessible to the males. At least, it suggests that this was a pharmacological effect of norepinephrine.

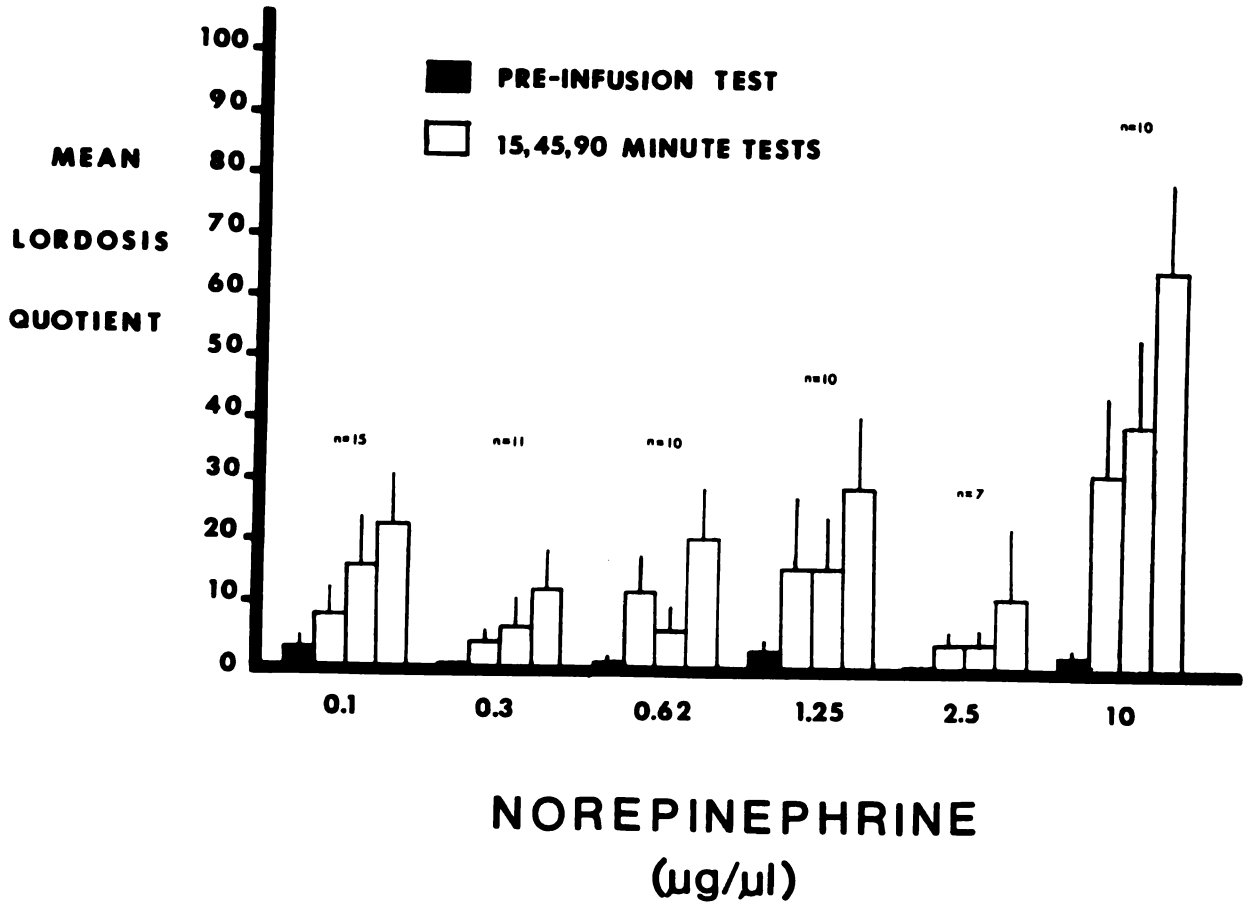

FIGURE 9

Lordosis behavior after medial preoptic infusion of six doses of norepinephrine (0.1 - 10 µg/µl) into ovariectomized estrogen-treated rats. All animals were injected with benzoate only (0.5 µg 72, 48 and 24 hours before testing). Each animal was tested for two weeks receiving two NE doses. These incomplete blocks in whole plots were subjected to a four-way ANOVA which found no significant effect of NE dose (see Appendix D). Histogram bars represent mean lordosis quotients, left to right 15, 45 and 90 minutes after infusion, for n animals at a particular dose. Lordosis quotients at the 90 minute tests were not significantly different from those for CSF vehicle infused animals (see Appendix D, Dunnett's t-tests).

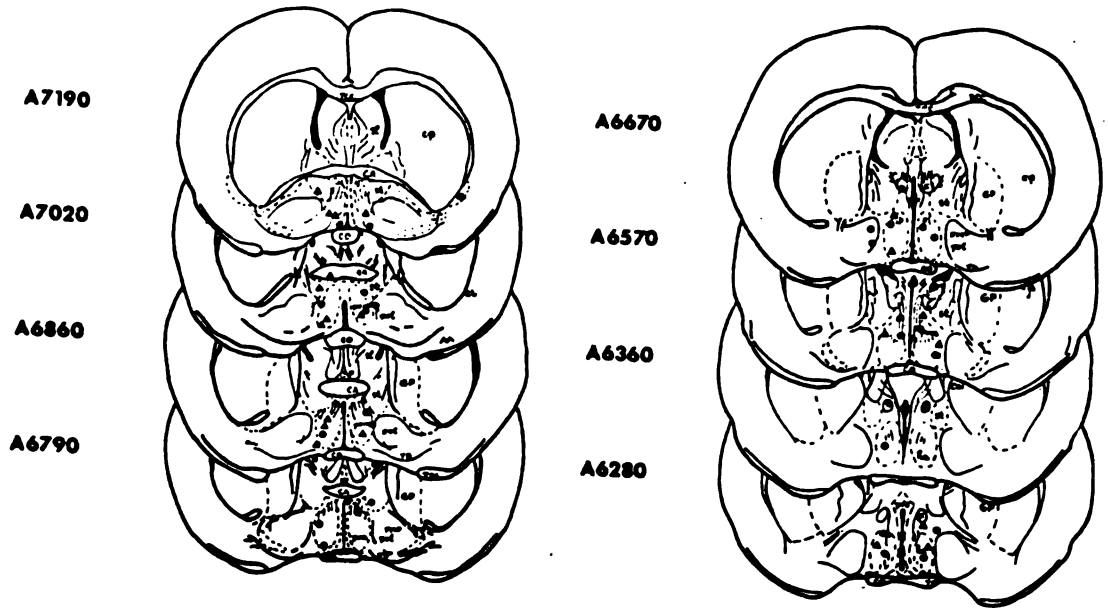

FIGURE 10

Analysis of these representative coronal slices (adapted from Konig & Klippel, 1963, by J. Kaminski) demonstrates that there was no clear localization of sites which resulted in a facilitation of lordosis. Estrogen-treated (0.5 µg EB for three days) animals were divided into those showing an increase in lordosis quotient of 50 or more from pre-infusion test levels to any post-infusion test (responders, solid circles) and those not showing LQ increases of 50 (non-responders, solid triangles).

Location of implant	# responders	# non-responders
MPOA sites	22	15
Non-MPOA sites	20	21

Fisher's test $F=1.81$, $p>.10$

Analysis of this distribution does not find significantly more responders to NE infusions in the MPOA than in other brain sites.

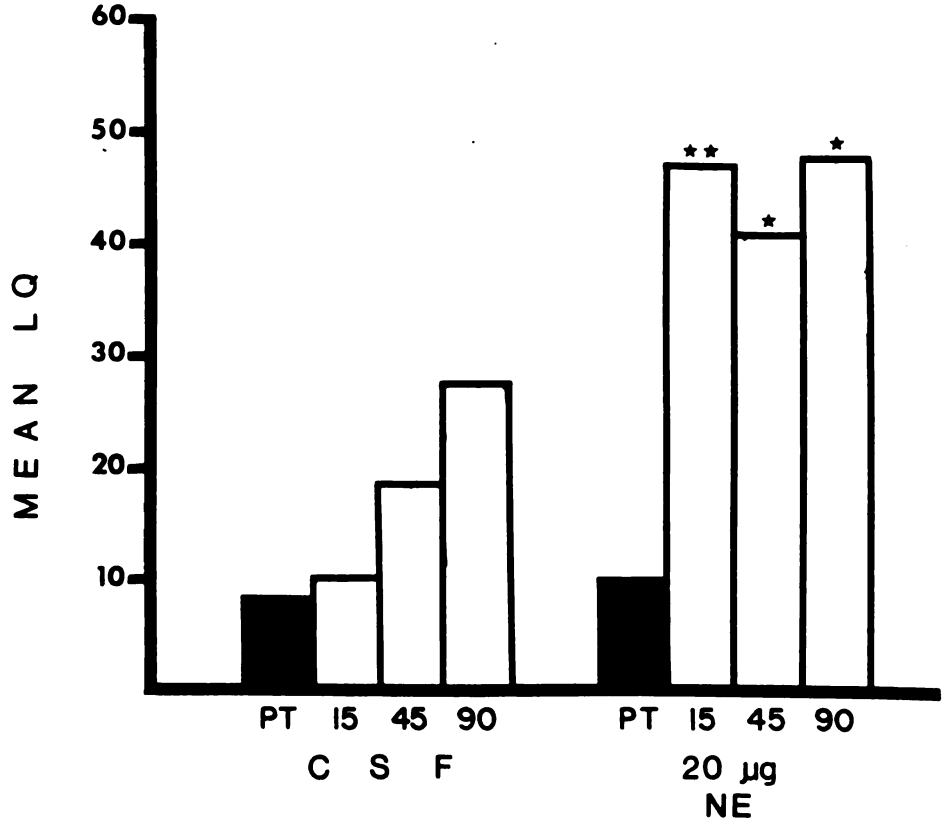

FIGURE 11

Infusion of 20 µg of norepinephrine into the medial preoptic area (MPOA) of eight estrogen-treated (0.5 µg EB for 3 days) ovariectomized female rats resulted in significant increases in receptivity at all post-infusion tests (Scheffe's interval tests: * = $p < .05$, ** = $p < .01$). Animals were infused with CSF alone or with 20 µg norepinephrine and were infused with the other treatment the second week. The ANOVA of this cross-over design had a significant effect of the NE dose (see Appendix E). Some of the NE infused animals demonstrated a general lethargy.

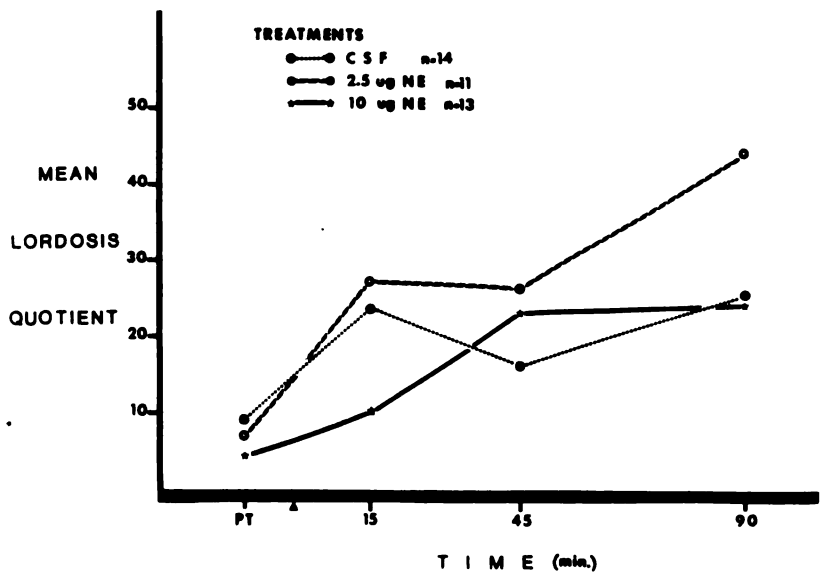

FIGURE 12

Norepinephrine infused into the MPOA did not enhance estrous behavior in animals treated with estrogen and progestrone (0.175 µg EB for 3 days and 500 µg progesterone on the fourth day 4-5 hours before testing). The three doses each animal received were CSF alone or with 2.5 or 10 µg norepinephrine. A Latin Square design was used to assign NE dose treatment order across the three weeks of testing. An analysis of variance found no significant effect of NE dose, however an overall increase in responding was seen across test times (see Appendix F).

Still the general increase in lordosis across time was curious. It appeared that repeated matings were increasing the probability of lordosis in later test sessions. If the act of mating served to release adrenal steroids, including progestins, this might account for such an increase across repeated matings. In the third design animals were injected adequate levels of progesterone but lower estrogen levels. It was assumed that, since animals already were given adequate progesterone levels, any norepinphrine-induced release of progestins would not affect receptivity. With this hormonal regime the increase across time was greatly reduced (see Figure 12). Again these data showed no significant effect of NE dose (see Appendix E, $F=1.26$, $d.f.=2,2$, $p>.25$) nor were the 90 minute test LQ scores significantly different from vehicle-infused animals (CSF vs. 2.5 ug $t_D=1.59$, $p>.05$; CSF vs. 10 ug NE $t_D=2.92$, $p>.05$). However, there was still a significant effect of time of observation (see Appendix E). This may indicate some small role of NE in increasing responding in the case where females are repeatedly tested. This increase in lordosis wiht repeated matings in estrogen-only treated animals may have masked any early inhibitory effects on norepinephrine.

Summary

There was no evidence to support the conclusion that norepinephrine acts endogenously to facilitate lordosis behavior. A rigorous analysis of lordosis responding after infusions of six norepinephrine doses found no dose-response relationship. A 20 ug dose of NE did significantly increase lordosis responding. However, the possibility that this was a pharmacological and not a physiological effect could not be discounted. Use of an hormonal regime of low estrogen and progesterone reduced the increase across repeated matings seen in animals treated with estrogen alone. This suggested that previously reported increases after infusion of noradrenergic agents may have partially been due to release of adrenal progestins.

DISCUSSION

Norepinephrine infused into the medial preoptic area inhibited lordosis behavior in five minutes. This short-latency reduction in lordosis frequency resulted after infusion of doses as low as 1 ug per cannula (2 ug/animal). Estrogen-progesterone treated receptive animals recovered from this inhibition in twenty minutes. Epinephrine infused at the same doses resulted in similar reductions in receptivity. The alpha-noradrenergic agonist clonidine, which is not as readily degraded as epinephrine or norepinephrine, continued to inhibit lordosis at 20 minutes. Also, higher doses of NE (10 and 20 ug/animal) inhibited lordosis responding up to 15 minutes after bilateral infusion. Attempts to counteract the effect of NE with specific receptor antagonists were frustrated by the transient nature of this inhibition. Only the alpha-noradrenergic antagonist yohimbine blocked the inhibitory action of the lowest effective dose of norepinephrine. $Alpha_1$-noradrenergic agonists methoxamine and phenylephrine did not reduce lordosis responding; neither did the beta-agonist isoproterenol. The inhibition of lordosis by norepinephrine was specific to the MPOA infusions. Norepinephrine infusions, by contrast, showed no capacity to induce receptivity in estrogen-treated unreceptive females.

The increase in lordosis seen with infusions of the 20 ug NE dose was thought to be pharmacological due to the lethargy it induced. There was, however, a consistent increase in lordosis frequency in vehicle-infused animals and across all NE doses across repeated matings. This increase across repeated matings was reduced by treating the animals with lower estrogen levels plus progesterone, but was not entirely eliminated. It thus appeared that the direct neurophysiological effect of norepinephrine on lordosis was inhibition and not facilitation.

Lordosis is an important and immediate motor response to specific somatic stimuli. Although ovarian hormones estrogen and progesterone prepare the neural substrate for this response, few believe that the role of these steroids is actually to be released from synaptic terminals in response to the somatosensory stimuli associated with mounting. These steroids are thought to utilize endogenous neurochemicals to translate the somatic stimulation into the ventral flexion that is the lordosis motor response. Such neurotransmitter action must necessarily be almost immediate. Any effect of infused substances which act as neurotransmitters would be expected to affect lordosis responding quickly. The short-latency effect of exogenously applied norepinephrine is a reduction in the frequency of the lordosis response to mounting by males.

The effect of infused norepinephrine is not only quick, it is transient. Presumably, estrogen and progesterone were still acting in these receptive animals to minimize lordosis-inhibitory influences and

maximize facilitative ones. If endogenous norepinephrine and/or epinephrine are inhibitory to lordosis then the steroids would maintain the catabolic enzymes to break them down. The exogenous flush of NE and epinephrine in infusions would therefore be expected to have only a transient effect. The inhibitory effect of the alpha$_2$-noradrenergic agonist clonidine had a longer duration. This may be because clonidine is not as readily metabolized by endogenous enzymes as are epinephrine and norepinrphrine. It may be that the longer the agent is capable of stimulating the alpha-receptor, the more the lordosis response is inhibited.

These data replicate the findings of Davis and Kohl (1977) who inhibited lordosis in rats by giving the alpha$_2$-agonist clonidine systemically and then blocked this effect with systemic yohimbine. Infusing these substances into the MPOA had similar effects. Experiment 3 localized at least one site of clonidine's inhibitory action. Because MPOA application is much nearer the site of action it is not surprising that the reduction was seen after 5 minutes instead of thirty minutes and at much smaller overall doses than with systemic treatment.

The inhibitory effectiveness of infused agents could be stated to be: clonidine > norepinephrine = epinephrine > phenylephrine > methoxamine = isoproterenol. Considering the nature of the behavioral assay this closely approximates the hierarchy of the binding affinities of these same agents for alpha$_2$-noradrenergic receptors (U'Prichard, Greenberg & Snyder, 1976; Berthelsen & Pettinger, 1977; U'Prichard & Snyder, 1978; Leibowitz et al., 1982). The only effective antagonist to

norepinephrine's inhibitory action was yohimbine which is believed to be specific to alpha$_2$ clonidine-binding receptors (U'Prichard & Snyder, 1978; Eriksson, Eden & Modigh, 1981; Leibowitz et al., 1982). Phentolamine, which is not effective, is not as specific to tritiated-clonidine binding sites as is yohimibine (U'Prichard, Greenberg & Snyder, 1976; Franklin & Herberg, 1977; U'Prichard & Snyder, 1978). It would appear that the inhibitory effects of NE infusions into the MPOA are mediated by alpha$_2$-receptors.

Several recent studies have found binding of tritiated clonidine and tritiated para-amino-clonidine, both specific for alpha$_2$receptors, in the medial preoptic area (Young & Kuhar, 1981; Leibowitz, 1982). Such alpha$_2$-receptors are thought to exist on presynaptic sites in the periphery (Starke, Endo & Taube, 1975; Strombom, 1975; Struyker-Boudier et al., 1975; Berthelsen & Pettinger, 1977; Langer, 1980). They also probably occur as autoreceptors in the locus coeruleus (Young & Kuhar, 1981; Aghajanian & VanderMaelen, 1982; Leibowitz, 1982). But alpha$_2$-receptors in other parts of the brain are not all presynaptic (Greenberg, U'Prichard & Snyder, 1976; U'Prichard, Greenberg & Snyder, 1976; U'Prichard et al., 1979; U'Prichard et al., 1980). Many of these studies used tritiated clonidine binding in brain regions after noradrenergic lesion by 6-OHDA (see Background). After such destruction cortical tritiated-clonidine binding increases. U'Prichard estimates that in such tissue 80% of alpha$_2$-receptors are post-synaptic or extra-synaptic (personal communication). Unfortunately they have yet to do the work in hypothalamus. It is impossible to say on which sort of preoptic component exogenous norepinephrine or epinephrine is acting.

It could be affecting post-synaptic, pre-synaptic or even extra-synaptic sites. Possible post-synaptic sites include neurons containing other neurotransmitters and/or neuropeptides. Extra-synaptic sites might be glia cells, blood vessels or other preoptic elements. These alpha$_2$-receptors have been implicated in the control of several neuroendocrine systems and in temperature/ cardiovascular control (see Background). It is thus, not surprising that norepinephrine and/or epinephrine would have their inhibitory effects on receptivity via these alpha$_2$-receptors.

The inhibitory effect of NE infusions was localized to the MPOA. This area serves many reproduction control functions. Noradrenergic input to this area modulates GnRH and several other reproductive parameters. Control infusions of another putative neurotransmitter, 5-HT or serotonin, into this area at equal doses was ineffective in decreasing estrous behaviors. Control infusions into the medial basal hypothalamus (MBH) did not reduce receptivity. However, the cannula tracts of the MPOA implants traverse the lateral ventricles to a greater extent than do those of bilateral implants in the MBH. This may give infusates greater access to the ventricles when infused into the MPOA. In preliminary studies a higher dose of NE was needed to inhibit lordosis quotients when it was infused directly into the lateral ventricles than when it was put into the MPOA (unpublished data). However, the timing of the inhibitory effect for ventricular NE infusions was the same, so that a circumventricular site of NE action cannot be completely ruled out.

Infused norepinephrine quickly reduces lordosis levels. The release of endogenous NE would have just such a short-latency effect. Temporally, the infusion of exogenous NE resembles the effect of endogenous neurotransmitter. But does this exogenous infusion relate to the physiological function of endogenous norepinephrine? One suggestion is that the MPOA is an inhibitory center with respect to female sexual behavior (see Background). Norepinephrine infusions here may mimic electrical stimulation of this region, which can act to inhibit lordosis responding (Pfaff & Sakuma, 1979). Since neuronal elements of a part of the MPOA are larger in males than females (Gorski et al., 1978; Gorski et al., 1980), preoptic stimulation may be eliciting competitive masculine behaviors which reduce feminine responses. While there is some evidence that catecholamines facilitate masculine behavior (see Caggiula et al., 1979), there is evidence that intraventricular NE decreases rather than increases preoptic neural activity (Salzman & Beckman, 1980; Leung et al., 1981; see Myers, 1980 for review). The MPOA is important in a number of behaviors and physiological functions (see Background). Stimulation of any of these functions could be said to increase other behaviors which are competitive with lordosis. However, it is necessary to realize that cells and not pieces or units of behavior are being affected by such infusions. The intimation that somehow NE is initiating competitive behavioral responses has little more than heuristic value. Assignment of function to preoptic nuclei does not help determine the actual physiological effect of NE in the MPOA.

A more fruitful approach may be to look at what is known about endogenous NE activity in the MPOA during estrous behavior. Norepinephrine turnover is a better indicator of noradrenergic activity than norepinephrine levels. Unfortunately, all that is known about preoptic NE turnover is that it is high after ovariectomy and during proestrus and that after estrous behavior ends turnover is low (see Background). But it is not known whether NE turnover remains high until the end of estrus or drops off after the proestrus surge. Ovariectomized rats given no estrogen are unreceptive yet show very high preoptic NE turnover levels. This would indicate that high NE turnover levels are not enough to induce receptivity. Norepinephrine infusions actually inhibit lordosis. These findings are consistent with a drop in preoptic NE turnover after the proestrous peak. That is, norepinephrine turnover may surge during proestrus to induce the LH peak and initiate ovulation and then the preoptic NE stores are spent and not replenished (Beattie, Rogers & Soyka, 1972). This leaves NE turnover rates low during estrus itself. The proestrous surge of preoptic NE may inhibit the animals from showing estrous behavior too early. Then a drop in noradrenergic activity releases the animal from this inhibitory influence. Rebound from this inhibition may be slightly facilitative. Also this does not preclude the possibility that the proestrous NE surge activates secondary mechanisms which are of longer-latency effect and which themselves enhance lordosis behavior to a male's mount. Some of these mechanisms will be discussed later.

Another explanation of the inhibitory effect of norepinephrine is that catecholamines are, in general, activators of behavior and that lordosis is an immobile response. It has been suggested that masculine sexual behaviors are active or mobile and feminine behaviors, particularly lordosis, are basically inactive or immobile (Caggiula et al., 1979). It is true that after NE infusions many animals were quite active. This confirms other findings that show hyperactivity 5-15 minutes after infusions of 10 ug NE intracerebrally (Leung et al., 1981). It may be that this increase in mobility interfered with the animal's ability to show lordosis. However, although locomotor activity is often influenced by gross catecholamine manipulations, such effects are believed to be mediated by dopamine in the nigro-striatal system (see Roberts, Zis & Fibiger, 1975).

Unlike the rat, in the guinea pig increasing norepinephrine activity results in augmented estrous behavior. Injections of clonidine into estrogen-treated guinea pigs increased the per cent of animals showing lordosis to manual stimulation (Nock & Feder, 1979). Noradrenergic activity may also increase the number of estrogen-induced cytoplasmic progestin-receptors in the preoptic-anterior hypothalamic area (Nock, Blaustein & Feder, 1981). Since clonidine can reverse the reduction in cytoplasmic progestin receptors caused by DBH inhibitors, this effect may be mediated by alpha$_2$-receptors (Nock, Blaustein & Feder, 1981). In the guinea pig it appears that increased NE activity during proestrus augments the number of preoptic progestin receptors. Presumably progesterone arrives to translocate these receptors into the

nucleus and begin to effect estrous behavior. Once progesterone begins translocating receptors something needs to shut off progestin-receptor production. In other words, there needs to be a negative feedback effect of progesterone on the estrogen-norepinephrine synergism which produced the receptors. In fact, the addition of progesterone to estrogen-treated animals reduces norepinephrine synthesis (Beattie, Rogers & Soyka, 1972) and turnover (Wise, Rance & Barraclough, 1981). This may illustrate progesterone's capacity to shut off norepinephrine's turnover before behavioral estrus.

There is still the curiosity that alpha-stimulation seems to be inhibitory in rats and facilitative to lordosis in guinea pigs. It may simply be that the immediate effect of clonidine in guinea pigs is inhibitory but that after thirty minutes, when the animals were first tested, the guinea pigs were responding to the release from that inhibitory influence. However, guinea pigs and rats are different in their responses to several treatments. First, the guinea pig lordosis is held longer than that for a rat (Dempsey, Hertz & Young, 1936). Treatment with clonidine decreased the lordosis duration in guinea pigs (Nock & Feder, 1979). It may be that stimulation of $alpha_2$-receptors activates a mechanism which ends the lordosis. Increasing the activity of a "stop" mechanism might shorten a guinea pig lordosis duration and reduce lordosis frequency in the rat. Another difference between guinea pigs and rats is that while the vagino-cervical stimulation of mating increases the probability of subsequent lordosis in rats (Larsson, Feder & Komisaruk, 1974), it will decrease future lordosis responding in

guinea pigs (Goldfoot & Goy, 1970). It is possible that noradrenergic activity mediates the response to vagino-cervical stimulation (Komisaruk, personal communication). Were this the case, it would not be surprising that guinea pigs and rats reacted oppositely to noradrenergic stimulation since they react in the opposite way to cervical stimulation.

There are other explanations for the inhibitory effects of norepinephrine infusions. One possibility is that norepinephrine decapacitates the animals so severely that they cannot show the lordosis motor response. In estrogen-progesterone animals there is no evidence that the animals were lethargic or debilitated except at the highest NE doses. However, in tests of motor function none of these animals showed any impairment (unpublished observations). There was no correlation between lethargy and low lordosis quotients. In estrogen-treated animals the higher doses (10 and 20 ug) resulted in some lethargy and even immobility. Such immobility appeared to have facilitated and not inhibited lordosis, perhaps by allowing the males easier access to the females. Release of adrenal progesterone in past studies has confused results using noradrenergic agents (see Background). However, the latency of an adrenal progesterone effect is thought to be around 4 hours (Feder & Ruf, 1969). These animals are already primed with adequate progesterone to induce receptivity. It is doubtful that further progestins, ACTH or other hormones would inhibit lordosis especially within 5 minutes. Another possibility is that NE infusions somehow temporarily lesion the MPOA. There is, however, no evidence

that MPOA lesion will reduce receptivity (Singer, 1968; Powers & Valenstein, 1972). If this fluid volume inhibits preoptic function then control CSF and saline infusions ought to have the same effect - they did not. It might be that the NE infusions lesioned neural tissue because of an unusual acid-base balance, but measurement of a 5 ug/ul dose of NE put its pH at 7.3 and infusion of a hydrochloric acid solution at this pH level had no inhibitory effect (unpublished data). Neither is the inhibition general to any infused neuroactive substance, since infusions of 5-HT were ineffective. The inhibitory effect is specific to norepinephrine. Such a specific temporary "lesion" seems much like neurotransmitter-induced inhibition.

Many early studies insisted that all noted inhibitory effects of monoamine manipulations were mediated by 5-hydroxytryptamine and facilitative effects mediated by norepinephrine. Direct infusions of these putative neurotransmitters into the MPOA provide no evidence to support either of these conclusions. No dose-response relationship was seen among six doses of NE and lordosis. Although a high dose of NE (20 ug) did increase lordosis quotients significantly, many of these animals were lethargic or immobile. This may indicate that 20 ug of NE results in a pharmacological rather than a physiological increase lordosis behavior. There was no clear localization of those infusions which caused increases in lordosis frequencies, further supporting the contention that NE plays no facilitative role in the control of estrous behavior. In vehicle-infused control animals and at all NE doses there was a significant effect of observation time. This probably reflects a

facilitative effect of repeated matings since it is even seen in CSF infused animals. It is difficult to determine if the addition of NE enhances this effect as it may have done in another study (Foreman & Moss, 1977).

Effects, such as this enhancement, which take over an hour to manifest themselves may also be due to any number of secondary effects of NE infusions. One suggested effect is an increase in neuropeptide release (Foreman & Moss, 1977). One intriguing neuropeptide is β-endorphin. Vaginal stimulation increases the probability of future lordosis behavior (Larsson, Feder & Komisaruk, 1974) and may increase noradrenergic activity (Komisaruk, personal communication). The noradrenergic system interacts with a discrete β-endorlphin system in the hypothalamus (Atweh & Kuhar, 1977; Aghajanian, 1978; Kuhar & Uhl, 1979; Strahlendorff, Strahlendorff & Barnes, 1980; Arnstein et al., 1981; Young & Kuhar, 1981). If norepinephrine enhances the activity of this β-endorphin system it could both block the aversive stimuli of male mounts and intromissions and reinforce the occurrence of vaginal stimulation. The existence of such a link is, however, highly speculative.

The more likely neurotransmitter role for preoptic norepinephrine is inhibition of lordosis behavior. Norepinephrine turnover in the MPOA increases during proestrus. This increase in turnover initiates the events which lead to ovulation. The arrival of quantities of progesterone into the area shuts down norepinephrine activity. The resulting drop in NE turnover then permits the expression of other

progesterone-initiated lordosis enhancing systems. The drop in NE turnover may even contribute to the enhancement of lordosis responding to mounting stimuli through a rebound from its inhibitory influence. Preoptic NE turnover remains low during behavioral estrus. During diestrus other inhibitory mechanisms act to maintain non-receptive behaviors.

Explaining a behavioral response such as lordosis in terms of turnover of one neurotransmitter in one brain nucleus is overly simplistic. The interaction between norepinephrine and the ovarian steroids is complex. Both preoptic neurons and noradrenergic neurons themselves sequester estrogen (Stumpf, 1968; Heritage et al., 1980). Estrogen decreases NE turnover in ovariectomized animals but helps create the proestrous increase in NE turnover (see Background). Microiontophoresis of norepinephrine into adjacent hypothalamic nuclei can result in opposite effects on the animal's endocrine response (Parvizi & Ellendorff, 1982). So there is not one monolithic response by the hypothalamus to noradrenergic input. Even in a single area like the MPOA multi-unit recordings will show differential responses of groups of neurons to norepinephrine and many of these groups respond differently depending on the hormonal environment (Leung et al., 1981). In this one small brain area norepinephrine has been associated with control of sleep, activity, GnRH, GH, TRF, temperature regulation, blood pressure and male and female reproductive behavior (see Background). Clearly much needs yet to be done to understand the workings of this brain area, this neurotransmitter and the interaction of them both with

ovarian hormones to control reproduction.

SUMMARY

Reductions in lordosis behavior are seen within five minutes of norepinephrine or epinephrine infusions into the medial preoptic area. Such reductions lasted to twenty minutes with infusions of the alpha$_2$-agonist clonidine into the same area. No such direct, physiological enhancement of lordosis was seen in unreceptive animals infused with norepinephrine. It is speculated that a drop in preoptic norepinephrine turnover rates after the proestrous surge permits the facilitative influence of progesterone to initiate estrous behaviors. Thus a surge in norepinephrine could be necessary to the occurrence of normal estrous behavior without actually being a facilitative influence on such behaviors.

LIST OF REFERENCES

References

Advis, J. P., S. M. McCann and A. Negro-Vilar. 1980. Evidence that catecholaminergic and peptidiergic (Luteinizing Hormone-Releasing Hormone) neurons in Suprachiasmatic-Medial Preoptic, Medial Basal Hypothalamus and Median Eminence are involved in estrogen-negative feedback. Endocrinology, 107,892-901

Aghajanian, G. K. 1978. Tolerance of locus coeruleus neurones to morphine and suppression of withdrawal response by clonidine. Nature (Lond.) 276,186-188

Aghajanian, G. K. and C. P. VanderMaelen. 1982. A_2-adrenoceptor-mediated hyperpolarization of locus coeruleus neurons: Intracellular studies *in vivo*. Science, 215,1394-1396

Ahlenius, S., J. Engel, H. Eriksson, K. Modigh and P. Sodersten. 1972a. Importance of central catecholamines in the mediation of Lordosis Behaviour in ovariectomized rats treated with estrogen and inhibitors of monoamine synthesis. J. of Neural Transmission, 33,247-255

Ahlenius, S., J. Engel, H. Eriksson and P. Sodersten. 1972b, Effects of tetrabenazine on Lordosis Behaviour and on brain monoamines in the female rat. J. of Neural Transmission, 33,155-162

Ahlquist, R. P. 1948. A study of the adrenotropic receptors. Am.j. of Physiology 153,586-600

Ambach, G. and M. Palkovitz. 1979. The Blood Supply of the Hypothalamus in the rat. In Handbook of the Hypothalamus Vol. 1 Anatomy of the Hypothalamus, edited by P. J. Morgane and J. Panksepp. Marcel Dekker, Inc. pp. 267-378

Anton-Tay, F., R. W. Pelham and R. J. Wurtman. 1969. Increased turnover of tritiated-norepinephrine in rat brain following castration or treatment with ovine follicle-stimulating hormone. Endocrinology 84,1489-1492

Arnstein, A. F. T., D. S. Segal, S. E. Laughlin and D. C. S. Roberts. 1981. Evidence for an interaction of opioid and noradrenergic locus coeruleus systems in the regulation of environmental stimulus-directed behavior. Brain Research, 222,351-363

Atweh, S. F. and M. J. Kuhar. 1977. Autoradiographic localization of opiate receptors in the rat brain. II. The Brain Stem. Brain Research 129,1-12

Axelrod, J. 1957. O-methylation of epinephrine and other catechols in vitro and in vivo. Science, 126,400-401

Barry, J. and M. P. DuBois. 1973. Etude en immunofluorescence des structures hypothalamique a competence gonadotrope. Annales Endocrinologiques, 34,735-742

Beach, F. A. 1942. Importance of progesterone to induction of sexual receptivity in spayed female rats. Proc. Society Exp. Biol. Med., 51,369-371

Beattie, C. W., C. H. Rogers and L. F. Soyka. 1972. Influence of ovariectomy and ovarian steroids on hypothalamic tyrosine hydroxylase activity in the rat. Endocrinology 91,276-279

Bereiter, D. A. and D. J. Barker. 1980. Hormone-induced enlargements of receptive fields in trigeminal mechanoreceptive neurons. 1. Time-course, hormone, sex and modality specificity. Brain Research, 184,395-410

Berk, M. L. and J. A. Finkelstein. 1981. Afferent projections to the preoptic area and hypothalamic regions in the rat brain. Neuroscience, 6,1601-1624

Berthelsen, S. and W. A. Pettinger. 1977. A functional basis for classification of α-adrenergic receptors. Life Sciences, 21,595-606

Biegon, A., H. Berkowitz and D. Samuel. 1980. Serotonin receptor concentration during the estrous cycle of the rat. Brain Research, 187,221-225

Blaschko, H. and H. Schlossmann. 1936. Decomposition of adrenaline in tissue. Nature, 137,110

Blaustein, J. D. and H. H. Feder. 1980. Nuclear progestin receptors in guinea pig brain measured by an in vitro exchange assay after hormonal treatments that affect lordosis. Endocrinology, 106,1061-1069

Blaustein, J. D., H. I. Ryer and H. H. Feder. 1980. A sex difference in the progestin receptor of guinea pig brain. Neuroendocrinology, 31,403-409

Blaustein, J. D., S. D. Dudley, J. M. Gray, E. J. Roy and G. N. Wade. 1979. Long-term retention of estradiol by brain cell nuclei and female rat sexual behavior. Brain Research, 173,355-359

Bleier, R., P. Cohn and I. R. Siggelkow. 1979. A cytoarchitectonic atlas of the hypothalamus and hypothalamic third ventricle of the rat. In Handbook of the Hypothalamus vol. 1 Anatomy of the hypothalamus. edited by P. J. Morgane and J. Panksepp. Marcel Dekker, New York pp.137-220

Boling, J. L. and R. J. Blandau. 1939. The estrogen-progesterone induction of mating responses in the spayed female rat. Endocrinology, 25,359-364

Boulant, J. A. 1980. Hypothalamic control of thermoregulation: Neurophysiological Basis. In Handbook of the Hypothalamus Vol. 3 Part A, Behavioral studies of the Hypothalamus. edited by P. J. Morgane and J. Panksepp, Marcel-Dekker, New York pp. 1-82

Bueno, J. and D. W. Pfaff. 1976. Single unit recording in hypothalamus and preoptic area of estrogen-treated and untreated ovariectomized female rats. Brain Research, 101,67-78

Byne, W., R. Bleier and I. Siggelkow. 1982. Hypothalamic sexual dimorphisms in four rodent species. Presented at the Society for Neuroscience Conference, Oct. 31-Nov. 5, Minneapolis,

Caggiula, A. R., J. G. Herndon, R. Scanlon, D. Greenstone, W. Bradshaw and D. Sharp. 1979. Dissociation of active from immobility components of sexual behavior in female rats by central 6-hydroxydopamine: Implications for catecholamine involvement in sexual behavior and sensorimotor responsiveness. Brain Research, 172,505-520

Carter, C. S., L. G. Clemens and D. J. Hoekema. 1972. Neonatal androgen and adult sexual behavior in the golden hamster. Physiology and Behavior, 9,89-95

Chambers, J. W. and G. M. Brown. 1976. Neurotransmitter regulation of growth hormone and ACTH in the rhesus monkey: Effects of biogenic amines. Endocrinology, 98,420-428

Christensen, L. W., D. M. Nance and R. A. Gorski. 1977. Effects of hypothalamic and preoptic lesions on reproductive behavior in male rats. Brain Res. Bulletin, 2,137-141

Clemens, L. G. 1971. Perinatal hormones and the modification of adult behavior. Chapter in Steroid Hormones and Brain Function, edited by C. H. Sawyer and R. A. Gorski, UCLA Medical Forum Series, University of California Press

Cohen, R. S. and D. W. Pfaff. 1982. Estrogen alters nerve cell nucleoli in rat hypothalamus. Presented at Society for Neuroscience Conference, Oct. 31-Nov. 5, Minneapolis

Conrad, L. C. A. and D. W. Pfaff. 1975. Axonal projections of medial preoptic and anterior hypothalamic neurons. Science, 190,1112-1114

Costa, E. 1973. Pharmacological implications of brain monoamie turnover rates elicited by (+) amphetamine and some chemically related compounds. In Psychopharmacology, Sexual disorders and Drug Abuse, edited by T. A. Ban, J. R. Boisier, G. J. Gessa, H. Heimann, L. Hollister, H. E. Lehmen, I. Munkvad, H. Steinberg, F.

Salser, A. Sundwall and O. Vinar North Holland, Amsterdam, pp. 637-658

Crowley, W. R., H. H. Feder and L. P. Morin. 1975. Role of monoamines in sexual behavior of the female guinea pig. Pharm. Biochem. & Behavior 4,67-71

Crowley, W. R., B. L. Nock and H. H. Feder. 1977. Facilitation of lordosis behavior by clonidine in female guinea pigs. Pharm. Biochem. & Behavior, 8,207-209

Dahlstrom, A. and K. Fuxe. 1964. Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brainstem neurons. Acta Physiol. Scand., 61(Suppl. 232), 1-55

Dahlstrom, A. and K. Fuxe. 1965. Evidence for the existence of monoamine neurons in the central nervous system IV. Distribution of monoamine nerve terminals in the central nervous system. Acta Physiol. Scand., (Suppl. 247),39-61

Davis, G. A. and E. English. 1977. Monoamines and lordosis behavior in the female guinea pig. Hormones & Behavior, 8,88-93

Davis, G. A. and R. Kohl. 1977. The influence of alpha-receptors on lordosis in the female rat. Pharm. Biochem. Behavior, 6,47-53

Davidson, J. M., E. R. Smith, C. H. Rogers and G. J. Bloch. 1968. Stmulation of female sex behavior in adrenalectomized rats with estrogen alone. Endocrinology, 82,193-195

Day, T. A., J. O. Willoughby and L. B. Geffen. 1979. Thermoregulatory effects of preoptic injections of noradrenaline in restrained and unrestrained rats. Brain Research, 174,175-179

Dempsey, E. W., R. Hertz and W. C. Young. 1936. The experimental induction of oestrous (sexual receptivity) in the normal and ovariectomized guinea pig. Am. j. Physiology, 116,201-220

Dohanich, G. P., J. A. Witcher, D. R. Weaver and L. G. Clemens. 1982. Alteration of muscarinic binding in specific brain areas following estrogen treatment. Brain Research, 241,347-350

Drouva, S. V. and R. V. Gallo. 1976. Catecholamine involvement in episodic luteinizing hormone release in adult ovariectomized rats. Endocrinology, 99,651-658

Durand, D., J. B. Martin and P. Brezeau. 1978. Evidence for a role of alpha-adrenergic mechanisms in the regulation of episodic growth hormone secretion in the rat. Endocrinology, 100,722-728

Dyer, R. G. 1974. The electrophysiology of the hypothalamus and its endocrinological implications. Prog. Brain Research, 41,133-147

Edwards, D. A., R. E. Whalen and R. D. Nadler. 1968. Induction of estrus: Estrogen-progesterone interactions. Physiol. & Behavior, 3,29-33

Eliasson, M., A. Michanek and B. J. Meyerson. 1972. A differential inhibitory action of LSD and amphetamine on copulatory behavior in the female rat. Acta Physiol. et Toxicol., 33, Suppl. I Abstract 22

Elde, R. and T. Hokfelt. 1979. Localiztion of hypophysiotropic peptides and other biologically active peptides in the brain. Ann. Rev. of Physiology, 41,587-602

Eriksson, H. and P. Sodersten. 1973. A failure to facilitate lordosis behavior in adrenalectomized and gonadectomized estrogen primed rats with monoamine-synthesis inhibitors. Hormones & Behavior, 4,89-97

Eriksson, E., S. Eden and K. Modigh. 1981. Importance of norepinephrine A_2-receptor activation for morphine-induced rat growth hormone secretion. Neuroendocrinology, 33,91-96

Estes, K. S., J. W. Simpkins and S. P. Kalra. 1982. Resumption with clonidine of pulsatile LH release following acute norepinephrine depletion in ovariectomized rats. Neuroendocrinology, 35,56-62

Everitt, B. J., K. Fuxe, T. Hokfelt and G. Jonsson. 1975. Role of monoamines in the female rat. J. of Comparative & Physiol. Psych., 89,556-572

Everitt, B. J., K. Fuxe and G. Jonsson. 1975. 5,7 Dihydroxy-tryptamine and the behavior of female rats. J. Pharmacologique de 1975, 6,25-32

Feder, H. H. and K. B. Ruf. 1969. Stimulation of progesterone release and estrous behavior by ACTH in ovariectomized rodents. Endocrinology, 84,171-174

Foreman, M. M. and R. L. Moss. 1978. Role of hypothalamic alpha- and beta-adrenergic receptors in the control of lordosis behavior in the ovariectomized rat. Pharm. Biochem. & Behavior, 9,235-241

Franklin, K. B. J. and L. J. Herberg. 1977. Presynaptic A-adrenoceptors: The depression of self-stimulation by clonidine and its restoration by piperoxane but not by phentolamine or phenoxybenzamine. Euro. j. of Pharm., 43,33-38

Frazer, M. S., D. R. Pieper, S. A. Tonetta, J. A. Duncan and J. C. Marshall. 1981. Pituitary gonadotropin-releasing hormone receptors: Effects of castration, steroid replacement, and the role of gonadotropin-releasing hormone in modulating receptors in the rat. J. of Clin. Investigations, 67,615-623

Freeman, W. J. and D. D. Davis. 1959. Effects on cats of conductive hypothalamic cooling. Am. j. of Physiology, 197,145-149

Gallo, R. V. and S. V. Drouva. 1979. Effect of intraventricular infusion of catecholamines on luteinizing hormone release in ovariectomized, steroid-primed rats. Neuroendocrinology, 29,149-162

Ganong, W. F. 1977. Neurotransmitters in ACTH secretion: Catecholamines. Annals of New York Acad. of Sciences. Science, 2997,509-517

Gay, V. L. and A. R. Midgely, Jr. 1966. Response of the adult rat to orchidectomy and ovariectomy as determined by LH radioimmunoassay. Endocrinology, 84,1359-1364

Gill, J. L. 1978. Design and Analysis of Experiments in Animal and Medical Experiments Vol. 2, Iowa State Univ. Press, Ames, Iowa

Goldfoot, D. A. and R. W. Goy. 1970. Abbreviation of behavioral estrus in guinea pigs by coital and vagino-cervical stimulation. J. of Comp. & Physiol. Psych., 72,426-434

Gorski, R. A., J. H. Shryne, F. Shapiro and A. M. Southam. 1978. Evidence for a morphological sex difference within the medial preoptic area of the rat. 148,333-336

Gorski, R. A., J. H. Gordon, J. E. Shryne and A. M. Southam. 1980. Evidence for the existence of a sexually dimorphic nucleus in the preoptic area of the rat. J. of Comp. Neurology, 193,529-539

Greenberg, D. A., D. C. U'Prichard and S. H. Snyder. 1976. Alpha-noradrenergic receptor binding in mammalian brain: Differential labeling of agonist and antagonist states. Life Sciences, 19,69-76

Gurdjian, E. S. 1927. The diencephalon of the albino rat: Studies on the brain of the rat #2. J. of Comp. Neurol., 43,1-114

Hanke, W. 1976. Neuroendocrinology. In Frog Neurobiology, edited by R. Llinas and W. Precht, Springer-Verlag, Berlin, pp. 975-1020

Hart, B. L. 1969. Gonadal hormones and sexual reflexes in the female rat. Hormones & Behavior, 1,65-71

Heimer, L. and K. Larsson. 1966/67. Impairment of mating behavior in male rats following lesions in the preoptic-anterior hypothalamic continuum. Brain Research, 3,248-263

Heritage, A. S., W. E. Stumpf, M. Sar and L. D. Grant. 1980. Brainstem catecholamine neurons are target sites for sex steroid hormones. Science, 207,1377-1379

Herndon, J. G., Jr. 1976. Effects of midbrain lesions on female sexual behavior in the rat. Physiol. & Behavior, 17,143-148

Herndon, J. G., A. R. Caggiula, D. Sharp, D. Ellis, E. S. Redgate, and M. J. Zigmond. 1978. Selective enhancement of the lordotic component of female sexual behavior in rats following destruction of central catecholamine-containing systems. Brain Research, 141,1148-1152

Herndon, J. G., A. R. Crowley, D. Sharp, D. Ellis and P. Redgate. 1978. Selective enhancement of the lordotic component of female sexual behavior in rats following destruction of central catecholamine-containing systems. Brain Research, 141,137-151

Hess, W. R. 1954. Diencephalon, Autonomic and Extrapyramidal functions, Grune & Stratton, New York

Hess W. R. 1957. The Functional Organization of the Diencephalon., Grune & Stratton, New York

His, W. 1893. Vorschlage zur eintheilung des gehirns. Arch. Anat. Physiol. Abt.,172-179

Hohn, K. G. and W. O. Wuttke. 1978. Changes in catecholamine turnover in the anterior part of the mediobasal hypothalamus and the medial preoptic area in response to hyperprolactonemia in ovariectomized rats. Brain Research, 156,241-252

Hokfelt, T., K. Fuxe, M. Goldstein and O. Johnsson. 1974. Immunohistochemical evidence for the existence of adrenaline neurons in the rat brain. Brain Research, 66,235-251

Hollander, M. and D. A. Wolfe. 1973. Nonparametric Statistical Methods, Wiley, New York

Honma, K. and W. Wuttke. 1980. Norepinephrine and dopamine turnover rates in the medial preoptic area and the medialbasal hypothalamus of the rat brain after various endocrinological manipulations. Endocrinology, 106,1848-1853

Jacobsen, C. D. and R. A. Gorski. 1981. Neurogenesis of the sexually dimorphic nucleus of the preoptic area in the rat. J. of Comp. Neurology, 196,519-529

Jacobsen, C. P., J. E. Shryne, F. Shapiro and R. A. Gorski. 1980. Ontogeny of the sexually dimorphic nucleus of the preoptic area. J. of Comp. Neurology, 193,541-548

Jorgensen, C. B. and L. O. Larsen. 1967. Neuroendocrine mechanisms in lower vertebrates. In Neuroendocrinology vol.2, edited by L. Martin and W. F. Ganong, New York Academy Press, pp. 485-528

Kalra, S. P. and S. M. McCann. 1973. Effects of drugs modifying catecholamine synthesis on plasma LH release induced by preoptic stimulation in the rat. Endocrinology, 93,356-362

Kalra, S. P. and S. M. McCann. 1974. Effects drugs modifying catecholamine synthesis on plasma LH and ovulation in the rat. Neuroendocrinology, 15,79-91

Kelley, D. B., J. I. Morrell and D. W. Pfaff. 1975. Autoradiographic localization of hormone-concentrating cells in the brain of an amphibian, Xenopus laevis I. Testosterone, J. of Comp. Neurology, 164,47-61

King, R., J. B. D. Samjen, A. M. Kaye and H. R. Linduer. 1979. Stimulation by estradiol-17β of specific cytoplasmic and chromosomal protein synthesis in immature rat uterus. Mol. Cell. Endocrinology, 1,21-36

Knigge, K. M., G. E. Hoffman, S. A. Joseph, D. E. Scott, C. D. Sladeck and J. R. Sladeck, Jr. 1980. Recent advances in structure and function of the endocrine hkypothalamus. In Handbook of the HYpothalamus Vol. 2, Physiology of the Hypothalamus, Edited by P. J. Morgane & J. Panksepp, Marcel Dekker Inc. New York, NY pp. 63-164

Komisaruk, B. R., N. T. Adler and J. Hutchison. 1972. Genital sensory field: Enlargement by estrogen treatment of rats. Science, 178,1925-1928

Konig, J. F. R. and R. A. Klippel. 1963. The Rat Brain: A stereotaxic atlas of the Foremrain and lower parts of the Brain Stem., Robert A.
Krieger Inc., Huntington, NY

Kow, L. M. and D. W. Pfaff. 1976. Sensory requirements for the lordosis reflex in female rats. Brain Research, 101,47-66

Krieg, R. J. and C. H. Sawyer. 1976. Effects of intraventricular catecholamines on luteinizing hormone release in ovariectomized-steroid-primed rats, Endocrinology, 99,411-419

Krulich, L., M. Quijada, E. Hefio and D. K. Sundberg. 1974. Localization of the thyrotropin releasing factor (TRF) in the hypothalamus of the rat. Endocrinology, 95,9-17

Kuhar, M. J. and G. R. Uhl. 1979. Histochemical localization of opiate receptors and the enkephalins. In Neurochemical Mechanisms of opiates and Endorphins (Adv. Biochem. Psychopharm. vol. 20), Edited by H. H. Loh and D. H. Ross., Raven Press, New York

Langer, S. Z. 1974. Presynaptic regulation of catecholamine release. Biochem. Pharmacology, 23,1793-1800

Larsson, K., H. H. Feder and B. R. Komisaruk. 1974. Role of the adrenal glands, repeated matings and monoamines in lordosis behavior of rats. Physiol. Biochem. & Pharm., 2,685-692

Leiberburg, I., N. MacLusky and B. S. McEwen. 1980. Cytoplasmic and nuclear estradiol-17β binding in male and female rat brain: Regional distribution, temporal aspects and metabolism. Brain Research, 193,487-503

Leibowitz, S. F. 1980. Neurochemical systems of the hypothalamus. In Handbook of the Hypothalamus, vol. 3 part A Behavioral studies of the hypothalamus, Edited by J. P. Morgane and J. Panksepp., Marcel Dekker Inc., New York pp. 299-408

Leibowitz, S. F., M. Jhanwar-Uniyal, b. Dvorkin and M. H. Maynard. 1982. Distribution of α-adrenergic and β-adrenergic and dopaminergic receptors in discrete hypothalamic areas of rat. Brain Research, 233,97-114

Leung, P. C. K., G. W. Arendash, D. I. Whitmoyer, R. A. Gorski, and C. H. Sawyer. 1982. Differential effect of central adrenoceptor agonists on luteinizing hormone release. Neuroendocrinology, 34,207-214

Leung, P. C. K., D. I. Whitmoyer, G. W. Arendash and C. H. Sawyer. 1981. Effects of intraventricular norepinephrine on preoptic-anterior hypothalamic electrical activity in the freely-moving rat: Modulation by ovarian steroid hormones. Brain Research, 226,143-154 and of translocation of estrogen-receptor in rat pituitary and uterus. Endocrinology, 110,265-271

Lindvall, O. and A. Bjorklund. 1978. Organization of catecholamine neurons in the rat central nervous system. In Handbook of Psychopharmacology Vol. Chemical Pathways in the Brain, Edited by L. L. Iversen, S. D. Iversen and S. H. Snyder, Plenum Press, New York

Lisk, R. D. 1962. Diencephalic placement of estradiol and sexual receptivity in the female rat. Am. j. of Physiology, 203,493-496

Lofstrom, A., P. Eneroth, J. A. Gustaffsson and P. Skett. 1977. Effects of estradiol benzoate on catecholamine levels and turnover in discrete areas of the median eminence and the limbic forebrain and on plasma LH, FSH and prolactin concentrations in the ovariectomized female rat. Endocrinology, 101,1559

Loo, Y. T. 1931. The forebrain of the opossum, Didelphis virginia. J. of Comp. Neurology, 52,1-148

Luine, V. N., R. I. Khylchevskaya and B. S. McEwen. 1975. Effect of gonadal steroids on activities of monoamine oxidase and choline acetylase in rat brain. Brain Research, 86,293-306

MacLusky, N. J. and B. S. McEwen. 1978. Oestrogen modulates progestin receptor concentrations in some rat brain regions but not in others. Nature (Lond.), 274,276-278

Mandel, P., G. Mack and C. Goridis. 1975. Function of the central catecholaminergic neuron: Synthesis, release and inactivation of transmitter. In Catecholamines and Behavior Vol. 1 Basic Neurobiology, Edited by A. J. Friedhoff, Plenum Press, New York

Marrone, B. L. and H. H. Feder. 1977. Characteristics of *estradiol and *progestin uptake and effects of progesterone on *estrogen uptake in brain, anterior pituitary and peripheral tissues of male and female guinea pigs. Biol. of Reproduction, 17,42-57

Martin, J. B. 1976. Brain regulation of growth hormone secretion. In Frontiers of Neuroendocrinology, Edited by L. Martini and W. F. Ganong, Raven Press, New York, pp. 129-188

Mautner, W. 1964. Das raumliche bild des neurosekretorischen zwischenhernsystems und der portalen hypophysenfasse van Rana temporaria und einigen anderen anuran mit leibenbeobachtungen am portalen hypophysenkreislauf. Z. Zellforsch., 64,813-826

McBride, R. L. and J. Sutin. 1976. Projections of the locus coeruleus and adjacent pontine tegmentum in the cat. J. of Comp. Neurology, 165,265-284

Meyerson, B. J. 1964a. Central nervous monoamines and hormone induced estrus behaviour in the spayed rat. Acta Physiol. Scand. 63, (Suppl. 241)

Meyerson, B. J. 1964b. The effect of neuropharmacological agents on hormone-activated estrus behaviour in ovariectomised rats. Archives Int. Pharmaco., 150,4-33

Meyerson, B. J. 1968. Amphetamine and 5-HT inhibition of copulatory behaviour in the female rat. Ann. Med. exp. Fenn., 46,394-398

Meyerson, B. J. and T. Lewander. 1970. Serotonin synthesis inhibition and estrous behavior in female rats. Life Science, 9,661-671

Meyerson, B. J., M. Eliasson, L. Lindstrom, A. Michanek and A. Ch. Soderlund. 1973. Monoamines and female sexual behaviour. In Psychopharmacology, Sexual Disorders and Drug Abuse, Edited by T. A. Ban, J. R. Boisier, G. Gessa, H. Hessmann, L. Hollister, H. Lehmen, I. Munkvad, H. Steinberg, F. Salser, A. Sundwall and O. Vinar, North Holland, Amsterdam, pp. 463-472

Michanek, A. and B. J. Meyerson. 1977. A comparative study of different amphetamines on copulatory behavior stereotype activity in the female rat. Psychopharmacology, 53,175-183

Mizuno, N., C. D. Clemente and E. K. Sauerland. 1969. Fiber projections from rostral basal forebrain structures in the cat. Exp. Neurology, 25,220-227

Moguilewsky, M. and J-P. Raynaud. 1977. Estrogen-sensitive progestin-binding sites in the female rat brain and pituitary. Biol. of Reproduction, 17,165-175

Moguilewsky, M. and J-P. Raynaud. 1979. The relevance of hypothalamic and hypophyseal progestin receptor regulation in the induction of sexual behavior in the female rat. Endocrinology, 105,516-522

Moore, R. Y. and F. E. Bloom. 1978. Central catecholamine neuron systems: Anatomy and physiology of the dopamine system. Ann. Rev. of Neuroscience, 1,129-169

Morrell, J. I., D. B. Kelley and D. W. Pfaff. 1975. Autoradiographic localization of hormone-concentrating cells in the brain of an amphibian, Xenopus laevis.nod, II. Estradiol. J. of Comp. Neurology, 164,63-77

Muth, E. A., W. R. Crowley and D. J. Jacobowitz. 1980. Effect of gonadal hormones on luteinizing hormones in plasma and on choline acetyltransferase activity and acetylcholine levels in discrete nuclei of the rat brain. Neuroendocrinology, 30,329-336

Myers, R. D. 1980. Hypothalamic control of thermoregulation: Neurochemical mechanisms. In Handbook of the Hypothalamus, vol. 3 Part A Behavioral Studies of the Hypothalamus, Edited by P. J. Morgane and J. Panksepp, Marcel-Dekker, New York pp. 83-210

Myers, R. D. and T. L. Yaksh. 1969. Control of body temperature in the unanesthesized monkey by cholinergic and aminergic systems in the hypothalamus. J. of Physiology, 202,483-500

Nagatsu, T. 1973. Biochemistry of Catecholamines: The Biochemical Method, University Park Press, Baltimore

Nock, B. and H. H. Feder. 1979. Noradrenergic transmission and female sexual behavior of guinea pigs. Brain Research, 166,369-380

Nock, B., J. D. Blaustein and H. H. Feder. 1981. Changes in noradrenergic transmission alter the concentrations of cytoplasmic progestin receptors in hypothalamus. Brain Research, 207,371-396

Ojeda, S. R., A. Negro-Vilar and S. M. McCann. 1982. Evidence for involvement of α-adrenergic receptors in norepinephrine-induced prostaglandin E_2 and luteinizing hormone-releasing hormone release from the median eminence. Endocrinology, 110,409-412

O'Malley, B. M. and W. T. Schrader. 1976. The receptors of steroid hormones. Scientific American, 234,32-43

Parsons, B., N. J. MacLusky, L. Drey, D. W. Pfaff and B. S. McEwen. 1980. The temporal relationship between estrogen-inducible progestin receptors in the female rat brain and the time course of estrogen activation of mating behavior. Endocrinology, 107,774-779

Parsons, B. and B. S. McEwen. 1981. Sequential inhibition of sexual receptivity by progesterone is prevented by protein synthesis inhibitor and is not causally related to decreased levels of hypothalamic progestin receptors in the female rat. The j. of Neuroscience, 1,527-531

Palkovitz, M., M. Fekete, G. B. McKoun and J. P. Herman. 1977. Total and partial hypothalamic deafferentations for topographical identification of catecholaminergic innervation of certain preoptic and hypothalamic nuclei. Brain Research, 127,127-136

Parvizi, N. and F. Ellendorff. 1982. Further evidence for dual effects of norepinephrine on LH secretion. Neuroendocrinology, 35,48-55

Paul, S. M., J. Axelrod, J. M. Saavedra and P. Skolnick. 1979. Estrogen-induced efflux of endogenous catecholamines from the hypothalamus in vitro, Brain Research, 178.499-505

Pavlik, E. J. and P. B. Coulson. Modulation of estrogen receptors in four different target tissues: Differential effects of estrogen versus progesterone. J. of Steroid Biochemistry, 7,369-375

Pfaff, D. W. 1968. Uptake of *H-estradiol by the female rat brain. Endocrinology, 82,1149-1155

Pfaff, D. W. and Y. Sakuma. 1979. Facilitation of the lordosis reflex of female rats from the ventromedial nucleus of the hypothalamus. J. of Physiology, 288,189-202

Powers, J. B. 1972. Facilitation of lordosis in ovariectomized rats by intracerebral progesterone implants. Brain Research, 48,311-336

Powers, J. B. and E. S. Valenstein. 1972. Sexual receptivity: Facilitation by medial preoptic lesions in female rats. Science, 1003-1005

Rainbow, T. C., P. G. Davis and B. S. McEwen. 1980. Anisomycin inhibits the activation of sexual behavior by estradiol and progesterone. Brain Research, 195,548-555

Rao, P. D. P. and H. G. Hartig. 1974. Manoaminergic traits of the diencephalon and innervation of the pars intermedia in Rana temporaria, Cell Tissue Research, 151,1-26

Roberts, W. W. and R. D. Mooney. 1974. Brain areas controlling thermoregulatory grooming, prone extension, locomotion and tail vasodilation in rats. J. of Comp. and Physio. Psych., 86,470-480

Roberts, D. C. S., A. P. Zis and H. C. Fibiger. 1975. Ascending catecholamine pathways of amphetamine-induced locomotor activity: Importance of dopamine and apparent non-involvement of norepinephrine. Brain Research, 93,441-454

Rodriguez-Sierra, J. F., W. F. Crowley and B. R. Komisaruk. 1975. Vaginal stimulation in rats induces prolonged lordosis responsiveness and sexual receptivity. J. Comp. Physio. Psych., 8,79-85

Rodriguez-Sierra, J. F., W. F. Crowley and B. R. Komisaruk. Induction of lordosis responsiveness by vaginal stimulation in rats is independant of anterior and posterior pituitary hormones. Hormones & Behavior, 8,348-355

Sadleir, R. M. F. S. Cycles and Seasons. In Reproduction in Mammals Vol. 1 Germ Cells and Fertilization, Edited by C. R. Austin & R. V. Short, Cambridge University Press, pp. 85-102

Salzman, S. K. and A. L. Beckman. 1980. Effects of TRH, NE and Ach on rat hypothalamic thermosensitive neurons. Presented at the Society for Neuroscience Conference 10th Annual Meeting, Nov. 9-14, Cincinatti

Sarkar, D. K., S. A. Chiappa, G. Fink and N. M. Sherwood. 1976. Gonadotropin-releasing hormone surge in pro-oestrous rats. Nature (Lond.), 264,461-463

Savoy-Moore, R. T., N. B. Schwartz, J. A. Duncan and J. C. Marshall. 1981. Pituitary gonadotropin-releasing hormone receptors in proestrus: Effect of pentobarbital blockade of ovulation in the rat. Endocrinology, 109,1360-1364

Schwartz, N. B. 1973. Mechanisms controlling ovulation in small mammals. In Handbook of Physiology, Vol. II, Female Reproductive Systems, part 1., Edited by P. O. Greep, American Physiological Society, Washington,

Short, R. V. 1972. Role of hormones in sex cycles. In Reproduction in Mammals: Vol. III Hormones in Reproduction, Edited by C. R. Austin & R. V. Short, Cambridge University Press, Cambridge, pp. 85-102

Siegel, S. 1956. Nonparametric Statistical for the Behavioral Sciences, McGraw-Hill, New York

Singer, J. J. 1968. Hypothalamic control of male and female sexual behavior in female rats. J. of Comp. & Physiol. Psych., 66,738

Smith, A. P. 1973. Mechanisms involved in the release of noradrenaline from sympathetic nerves. Brit. Med. Bulletin, 29,123-129

Smith, A. P. 1979. Biochemical studies of the mechamism of release. In Release of Catecholamines from Adrenergic Neurons, Edited by D. M. Paton, Pergamon Press, Oxford

Sodersten, P. and S. Ahlenius. 1972. Female lordosis behavior in estrogen primed male rats treated with p-chlorophenylalanine or α-methyl-p-tyrosine. Hormones & Behavior, 3,181-189

Starke, K., T. Endo and H. D. Taube. 1975. Relative pre- and postsynaptic potencies of alpha-adrenoceptor agonists in the rabbit pulmonary artery. Naunyn-Schmeideberg's Arch. Pharmacol., 291,55-78

Stefano, F. J. E. and A. O. Donoso. 1967. Norepinephrine levels in the rat hypothalamus during the estrous cycle. Endocrinology, 81,1405-1406

Steinman, J. L., L. A. Roberts and B. R. Komisaruk. 1982. Evidence that endogenous opiates contribute to the mediation of vaginal stimulation produced antinociception in rats. Presented at Society for Neuroscience Conference 12th Annual Meeting, Oct. 31 - Nov. 5, Minneapolis

Strahlendorff, H. K., J. C. Strahlendorff and C. D. Barnes. 1980. Endorphin-mediated inhibition of locus coeruleus neurons. Brain Research, 191,284-288

Strombom, U. 1975. On the functional role of pre- and postsynaptic catecholamine receptors in brain. Acta Physiol. Scand., Suppl. 431

Struyker-Boudier, H., J. De Boer, G. Smeets, E. J. Lien and J. Van Rossum. 1975. Structure activity relationships for central and peripheral alpha adrenergic activities of imidazoline derivatives. Life Sciences, 17,377-386

Struyker-Boudier, H., G. W. M. Smeets, G. M. Brouwer J. M. Van Rossum. 1974. Hypothalamic alpha-adrenergic receptors in cardiovascular regulation. Neuropharmacology, 13,837-846

Stumpf, W. E. 1968. Estradiol-concentrating neurons: Topography in the hypothalamus in the hypothalamus by dry-mount autoradiography. Science, 162,1001-1003

Tima, L. and B. Flerko. 1974. Ovulation induced by norepinephrine in rats made anovulatory by various experimental procedures. Neuroendocrinology, 15,346-354

Toivola, P. and C. C. Gale. 1970. Effect on temperature of biogenic amine infusion into hypothalamus of baboon. Neuroendocrinology 6,210-219

Udenfriend, S. 1966a. Biosynthesis of the sympathetic neurotransmitter, norepinephrine. In The Harvey Lectures, Series 60, Academic Press, New York

Udenfriend, S. 1966b. Tyrosine hydroxylase. In Second Symposium on Catecholamines: Pharmacological Reviews, 18,43-51

Ungerstedt, U. 1971. Stereodtaxic mapping of the monoamine pathways in the rat brain. Acta Physiol. Scand., Suppl. 367,95-122

U'Prichard, D. C., W. D. Bechtel, B. M. Rouot and S. H. Snyder. 1979. Multiple apparent alpha-noradrenergic receptor binding sites in rat brain: Effect of 6-hydroxydopamine. Molecular Pharmacology, 16,47-60

U'Prichard, D. C., D. A. Greenberg and S. H. Snyder. 1976. Binding characteristics of a radiolabeled agonist and antagonist at central nervous system alpha noradrenergic receptors. Molecular Pharmacology, 13,454-473

U'Prichard, D. C.,T. D. Reisine, S. T. Mason, H. C. Fibiger and H. I. Yamamura. 1980. Modulation of rat α- and β-receptor populations by lesion of the dorsal noradrenergic bundle. Brain Research, 187,143-154

U'Prichard, D. C. and S. H. Snyder. 1979. Distinct α-noradrenergic receptors differentiated by binding and physiological relationships. Life Sciences, 24,79-88

Vale, W., C. Rivier and M. Brown. 1977. Regulatory peptides of the hypothalamus. Ann. Rev. of Physiol., 399,473-527

Van Loon, G. R. 1973. Brain catecholamines and ACTH secretion. In Frontiers in Neuroendocrinology 1973, Edited by W. F. Ganong and L. Martini, Oxford University Press, New York pp.209-248

Valverde, F. 1963. Studies on the forebrain of the mouse. Golgi observations. J. of Anatomy (Lond.), 97,157-180

Veale, W. L. and I. Q. Whishaw. 1976. Body temperature responses at different ambient temperatures following injections of prostaglandin E_1 and noradrenaline into the brain. Pharm. Biochem. & Behavior, 4,143-150

Vijayen, E. and S. M. McCann. 1978. Reevaluation of the role of catecholamines in the control of gonadotropin and prolactin release. Neuroendocrinology, 25,150-165

Ward, I. L., W. R. Crowley, F. P. Zemlan and D. L. Margules. 1975. Monoaminergic mediation of female sexual behavior. J. of Comp. and Physiol. Psych., 88,53-61

Ward, I. L., J. E. Franck and W. R. Crowley. 1977. Central progesterone induces female sexual behavior in estrogen-primed intact male rats. J. of Comp. and Physiol. Psych., 91,1417-1423

Weick, R. F. 1978. Acute effects of adrenergic receptor blocking drugs and neuroleptic agents on pulsatile discharge of luteinizing hormone in the ovariectomized rat. Neuroendocrinology, 26,108-117

Weiner, R. I. and F. Ellendorff. 1982. Further evidence on dual effects of norepinephrine on LH secretion. Physiological Reviews, 58,905-976

Weiner, R. I. and W. F. Ganong. 1978. Role of brain monoamines and histamine in regulation of anterior pituitary secretion. Phsyiological Reviews, 58,905-976

Wise, P. M., N. Rance and C. Barraclough. 1981. Effects of estradiol and progesterone on catecholamine turnover rates in discrete hypothalamic regions in ovariectomized rats. Endocrinology, 108,2186-2193

Yanase M. and R. A. Gorski. 1976. Sites of estrogen and progesterone facilitation of lordosis behavior in the spayed rat. Biol. of Reproduction, 15,536-543

Young, W. S., III and M. J. Kuhar. 1979. Noradrednergic alpha-1 and alpha-2 receptors: Autoradiographic visualization. Euro. j. or Pharmacology, 59,317-319

Zambrano, D. and E. Robertis. 1968. Ultrastructure of the peptidergic and monoaminergic neurons in the hypothalaic neurosecretory system of anuran batracians. Z. Zellforsch., 90,230-244

Zigmond, R. E. and B. S. McEwen. 1970. Selective retention of oestradiol by cell nuclei in specific brain regions of the ovariectomized rat. J. of Neurochemistry, 17,889-899

Zuckerman, M. 1971. Physiological measures of sexual arousal in the human. Psychol. Bulletin, 75,297-239

APPENDICES

APPENDIX A

Analysis of variance of norepinephrine inhibition at 10 and 20 µg doses infused into the MPOA of estrogen-progesterone treated ovariectomized rats

Source of Variance	Sum of Squares	Degrees of freedom	Mean Square	F=	Probability <
Square	.132	1	.132	18.92	.05
Animals/Square	.174	7	.0248	3.56	n.s.
Week (Square)	.269	4	.067	9.64	.05
Dose	.075	2	.037	5.34	n.s.
Animal X Week (Error 1)	.028	4	.0069	MS_{e1}	
Time	.714	21	.079	10.31	.001
Time (Animal)	.348	21	.016	2.15	.032
Time (Dose)	.274	6	.045	5.93	.0005
Error 2	.2	26	.0077	MS_{e2}	

Dunnett's T comparing 15 minute tests of:

CSF vs 10 µg T_D= 6.46, d.f. = 2.14; $p < .01$

CSF vs 20 µg T_D= 5.33, d.f. = 2.11; $p < .01$

APPENDIX B

Kruskal-Wallis, Dunn's nonparametric test, Mann-Whitney U and paired t-tests comparing reductions in LQ in the following groups to saline vehicle-infused controls

Infusate	NE dose	N=	Kruskal-Wallis	Dunn's test	p<	Mann-Whitney U	p<	paired t-test	p<
Saline		55	24.68 (p<.001)						
	0.5	9		3.96	ns	47	ns	1.78	ns
	1	8		9.64	ns	37	ns	2.93	.05
	2	14		21.4	.05	29	.01	5.36	.01
	5	7		21.9	.05	4	.01	4.43	.01
	10	5		26.8	.05	11	.05	3.91	.02
Yohimbine		43	18.6 (p<.01)						
	0.5	6		6.64	ns	27	ns	1.88	ns
	1	7		13.8	ns	18	.05	2.77	.05
	2	8		8.84	ns	36	ns	2.26	ns
	5	7		16.84	ns	13	.05	3.32	.02
	10	5		22.84	.01	0	.001	4.24	.02
Phentolamine		30	22.22 (p<.001)						
	0.5	5		0.9	ns	23	ns	0.92	ns
	1	7		9.07	ns	35	ns	2.12	ns
	2	7		15.86	ns	7	.001	3.43	.01
	5	6		21.16	.05	8	.01	4.6	.01
	10	5		18.78	ns	5	.01	3.14	.05

APPENDIX C

Analysis of variance for six norepinephrine doses (0.1-10 µg) infused into the MPOA of estrogen-treated unreceptive rats

Source of variance	Sum of Squares	degrees of freedom	Mean Square	F=	p<
Squares	2.48	12	.207	2.25	ns
Subjects	.185	3	.062	.675	ns
Weeks	.275	1	.275	2.99	ns
Doses	1.49	5	.3	3.26	ns
$Error_1$	.918	10	.09	MS_{e_1}	
Time	.377	2	.188	3.99	.001
Time x Dose	.123	10	.012	.26	ns
Time x Week	.006	2	.003	.063	ns
$Error_2$	7.52	159	.047	MS_{e_2}	

ns = not significant, p>.05

CSF versus:	0.1 µg	0.3 µg	0.62 µg	1.25 µg	2.5 µg	10 µg
Dunnett's T (m=6)	0.4	1.15	0.49	0.11	1.12	2.86
d.f.	21	17	16	16	13	16
	ns	ns	ns	ns	ns	ns
Mann-Whitney U	24	47	33	46	31	33
p<	.01	ns	ns	ns	ns	ns
n =	15	11	10	10	7	10

APPENDIX D

Analysis of variance of artificial cerebrospinal fluid infused controls versus 20 μg norepinephrine infused estrogen-treated animals

Sources of variance	Sum of Squares	degrees of freedom	Mean Square	F=	p<
Subjects	9.6	7	1.37	6.6	.008
Dose	6.08	1	6.08	22.9	.001
Time	.71	2	.355	1.33	ns
Subject x Dose	4.06	7	.581	2.19	ns
Subject x Time	5.2	14	.37	1.4	ns
Dose x Time	.3	2	.15	.568	ns
Time x Week	.2	2	.1	.39	ns
Residual	2.9	11	.265	MS_e	

ns = not significant, p>.05

Scheffe's interval test for CSF versus 20 μg at: 15 minutes q=3.62, d.f.=1, p<.01
45 minutes q=2.25, d.f.=1, p<.01
90 minutes q=2.25, d.f.=1, p<.01

Scheffe's interval by weeks: Week 1 q=12.35, Week 2 q=6.0
$q_{k\pm f}.01,6$= 1.35, p<.01 for both weeks

APPENDIX E

Analysis of variance in estrogen-(0.175 µg EB x 3 days) progesterone-treated rats infused with CSF, 2.5 or 10 µg norepinephrine

Sources of variance	Sum of Squares	Degrees of freedom	Mean Square	F=	p<
Square	10.2	1	10.2	1.9	ns
Animal (Square)·	105.8	7	15.1	2.82	ns
Week (Square)	65.9	4	16.48	3.08	ns
Dose	13.23	2	6.61	1.24	ns
Square x Dose	.258	1	.258	.048	ns
Time	112.2	9	12.47	3.47	.01
Time (Animal)	91.35	21	4.35	1.21	ns
Time (dose) Error $_1$	32.1	6	.35	.49	ns
Error$_2$	79.0	22	3.59	MS_{e2}	

ns = not significant, p>.05