This is to certify that the

thesis entitled

NEUROPHARMACOLOGICAL CONTROL OF SEXUAL
BEHAVIOR IN FEMALE RATS

presented by

Raymond R. Humphrys

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Zoology and
Neuroscience

Major professor

Date August 7, 1978

MSU

# NEUROPHARMACOLOGICAL CONTROL OF SEXUAL BEHAVIOR IN FEMALE RATS

By

Raymond Robertson Humphrys

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Zoology

1978

ABSTRACT

NEUROPHARMACOLOGICAL CONTROL OF SEXUAL BEHAVIOR IN FEMALE RATS

By

Raymond Robertson Humphrys

Neurotransmitters in the central nervous system have been implicated in the regulation of female sexual behavior. The nature of this regulation was investigated in a series of experiments. In Part A, it was found that implantation of cholinomimetics (carbachol and bethanechol), as well as an anticholinesterase compound (neostigmine bromide), facilitated lordosis frequency of ovariectomized, estrogen-primed female rats. Within the mesencephalic reticular formation (MRF), stimulation of two types of cholinergic receptors, muscarinic and nicotinic, increased lordosis frequency. In the medial preoptic area-anterior hypothalamus (MPOA-AH), only stimulation of muscarinic receptors increased lordosis frequency. That these facilitative effects were not the result of diffusion of the drug to other sites was indicated by the fact that implantation of carbachol into the posterior hypothalamus (PHA) was without effect. The facilitative effects seen with MPOA-AH implants cannot be attributed to the liberation of adrenal steroids, since adrenalectomized female rats continued to show increased lordotic frequencies in response to cholinergic stimulation.

The serotonergic systems have also been implicated in the mediation of female sexual behavior, and the possibility that serotonergic interactions with cholinergic systems within the MPOA were responsible for increasing the probability of lordotic behavior was examined in Part B. Inhibition of serotonergic neurotransmission (with methysergide) facilitated sexual behavior in estrogen-primed female rats, while enhancement of serotonergic neurotransmission (with serotonin, 5-HT) inhibited estrogen-progesterone-activated sexual behavior. Sequential inhibition of both serotonergic and nicotinic processes increased lordosis frequency. These results were taken as evidence that in the MPOA-AH of estrogen-primed female rats nicotinic and serotonergic systems exert a tonic inhibitory influence over the lordotic reflex. This interpretation was consistent with the failure to find a facilitation of female sexual behavior following nicotinic stimulation in Part A.

Part C was an investigation of the possible involvement of catecholaminergic systems in the mediation of female sexual behavior. Sequential administration of compounds which stimulate adrenergic (l-epinephrine), and block beta-adrenergic (LB-46), receptors, significantly facilitated lordosis when these compounds were implanted in the MPOA-AH. Another group of rats was pretreated with estrogen and progesterone. When compounds which potentiate adrenergic (norepinephrine; NE) and dopaminergic (dopamine; DA) neurotransmission were injected into the MPOA-AH, NE decreased the frequency of lordosis whereas DA was without effect. The results of the above studies suggest that, within the hypothalamus at least, there exists

a redundancy of lordosis-facilitative and -inhibitory circuits. This interpretation was based on: (1) the increase in lordosis frequency following stimulation of cholinergic and inhibition of serotonergic processes; (2) the decrease in lordosis frequency by enhancing serotonergic neurotransmission; and (3) the decrease in lordosis frequency by enhancing noradrenergic but not dopaminergic neurotransmission.

The data presented in this dissertation are consistent with the notion that: (1) the muscarinic and nicotinic cholinergic systems within the MRF are involved in the facilitation of sexual receptivity; and, (2) serotonergic, nicotinic (within the MPOA-AH), and noradrenergic neural systems are involved in regulating female sexual behavior, perhaps through an inhibitory mechanism.

DEDICATION

To Valerie and my parents with love

## ACKNOWLEDGMENTS

I would like to express my sincere appreciation to each of the members on my guidance committee: Dr. L. G. Clemens, Dr. Thomas W. Jenkins, Dr. John I. Johnson, and Dr. Richard H. Rech. To each I owe a special word of thanks for providing me with valuable and personal insights.

I would like to thank all of the past and present students in the Hormones and Behavior Laboratory: Dr. Joseph F. DeBold, M. John Dwyer, Brian A. Gladue, Teresa V. Popham, Patricia H. Ruppert, and Dr. Archie J. Vomachka.

This research was supported by U.S.P.H.S. Research Grant: H.D.-06760 to Dr. L. G. Clemens.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

# GENERAL INTRODUCTION

Sexual behavior is necessary for the survival of the species, and in most female vertebrates is a cyclical phenomenon regulated by rhythmic secretory patterns of pituitary and ovarian hormones. These repetitive hormonal changes result in the maturation of ovarian follicles, the release of ova, proliferation of the uterine endometrium, and the occurrence of behavioral receptivity. Because of the cyclic nature of female sexual receptivity, its synchronization with the sexual activity of the male is a primary requirement. The phase-matching of the female's cycle with that of the male's is achieved by the use of species-specific cues. These signals, which include visual, olfactory and auditory processes, are integrated into a complex species-specific behavioral pattern which serves as an isolating mechanism. These mechanisms function in isolating participants of the same species and in addition assures that each participant is in the appropriate reproductive condition. The integration of these complex physiological and behavioral mechanisms insures fertile mating and thus the preservation of the species.

The neuroanatomical and/or chemical substrates which subserve sexual behavior have been studied in several different species of mammals. These include rats, hamsters, guinea pigs, and mice. In studying female sexual behavior, the choice of the species is the most obvious organismic variable. Each of these species exhibits

a different pattern of mating behavior; therefore, this discussion will be limited to the rat unless otherwise noted. In addition, hormonal and environmental factors exert varying degrees of control depending on the species.

The importance of the ovarian hormones, estrogen and progesterone, for the display of female sexual behavior in non-primate species is well documented (see Young, 1961). During the afternoon of physiological estrus the female rat, guinea pig, mouse, or hamster willingly copulates with conspecific males (Young, 1961). This periodic appearance of sexual receptivity is directly related to the secretory cycle of the ovary; many investigators have demonstrated that administration of estrogen, or estrogen and progesterone, results in the appearance of sexual receptivity in the ovariectomized female rodent (e.g., Allen, 1924; Beach, 1942; Boling & Blandau, 1939; Dempsey, 1936; Young, 1961). While the specific details of the female rodent's copulatory pattern vary from one species to the response cannot be evoked during the other days of the cycle, nor is it observed in the hormonally-untreated ovariectomized female. occurs during copulation on the afternoon of estrus. This postural response cannot be evoked during the other days of the cycle, nor is it observed in the normonally-untreated ovariectomized female.

The specific physiological and anatomical bases for the actions of estrogen and the synergism of estrogen and progesterone are unknown at the present time. In the female rat, neural sites which have been implicated in the mediation of estrogen and progesterone's effects on sexual behavior are the medial preoptic

area-anterior hypothalamus (MPOA-AH) and the mesencephalic reticular formation (MRF). Autoradiographic studies using $^{3}$H-estradiol have demonstrated that the MPOA-AH, median eminance, amygdala, and septum possess high-affinity estradiol receptors which concentrate the tritiated steriod in the cell bodies and glial cells (e.g., Eisenfield & Axelrod, 1967; Jensen & Jacobson, 1962; Pfaff & Keiner, 1973; Stumpf, 1970). Similarly, autoradiographic studies in female rats, mice, and guinea pigs have shown that $^{3}$H-progestins are taken up and retained in the mesencephalon (Luttge et al., 1974; Whalen & Luttage, 1971). Additionally, direct intracerebral treatment with crystalline estrogens in the MPOA-AH increases lordotic responses in female cats and rats (Harris & Michael, 1964; Lisk, 1962), and intracerebral implants of progesterone in the MRF facilitate lordotic behavior (Clemens, 1972; Ross et al., 1971).

One hypothesis which is often used to explain how ovarian hormones facilitate sexual receptivity is that estrogen and progesterone synergize to decrease a state of tonic neural inhibition which is mediated by rostral brain areas, e.g., MPOA-AH, septum, and/or the anterior hypothalamus (see review, Clemens, 1978).

The present study was designed to provide information on the neuropharmacological mechanisms involved in the mediation of sexual receptivity in the ovarietomized female rat. The present experiments are based on the proposition that the effects of ovarian hormones upon sexual behavior are mediated by functional alterations in neurotransmitter activity. The involvement of neurotransmitters may be the result of a complex series of neuroendocrine interactions.

Thus, neurotransmitters may act to mediate sexual receptivity at one or more levels. For example, the neurotransmitters acetylcholine, norepinephrine, dopamine, and serotonin, as well as their anabolic and catabolic enzymes, are found in the estrogen-sensitive MPOA-AH and the progesterone-sensitive MRF (Fonnum et al., 1977). These substances have also been implicated in the more direct pituitary control of gonadal (Kordon & Glowinski, 1972), adrenal (Schaepdryer et al., 1969), and thyroid functions (Grimm & Reichlin, 1973). In the ovariectomized, estrogen-treated female rat, it has been shown that induction of sexual behavior (lordosis) is possible with systemic administration of compounds which deplete total concentrations of serotonin (5-HT), norepinephrine (NE), and dopamine (DA) (Ahlenius et al., 1972a, b; Everitt et al., 1975a, b), or stimulate cholinergic systems (Lindstrom, 1973; Lindstrom & Meyerson, 1967). Increases in lordotic responding have also been observed following direct central modifications of monoaminergic activity; e.g., depletion of NE and DA following intraventricular treatment with the neurotoxin 6-hydroxydopamine enhances lordosis frequency (Herndon et al., 1978).

The effects of estrogen and progesterone upon sexual behavior may be influenced by alterations in specific brain systems, e.g., the MPOA-AH and MRF. Thus, the nonspecific modification of neurotransmitter systems throughout the entire brain following systemic drug treatment may obscure differential functions of the transmitters within anatomically distinct brain regions. Therefore, the experiments reported here are based not only upon the hypothesis that

ovarian hormone effects upon female sexual behavior are mediated by functional alterations in neurotransmitter dynamics, but also that these effects are mediated by cells within specific areas of the brain. These experiments were designed to: (1) localize behaviorally effective sites of neurotransmitter action in the brain; and (2) determine the contributions of the cholinergic and monoaminergic systems in the MPOA-AH and the MRF in the regulation of female sexual behavior.

Part A was designed to: (1) determine whether cholinergic stimulation of the estrogen-sensitive MPOA-Ah and the progesterone-sensitive MRF would influence lordosis; (2) localize the effective sites of action; and (3) determine the contributions of muscarinic and nicotinic (systems) within these specific brain areas. Part B was designed to test the hypothesis that: (1) a serotonergic neuron within the POA-Ah exists that maintains a tonic inhibitory influence over lordosis; and (2) that mediation of sexual behavior involves a functional interaction between serotonergic and cholinergic neurons in the MPOA-AH. The purpose of Part C was to determine whether alpha- and beta-adrenergic systems participate in the regulation of sexual receptivity, and if the catecholamines, norepinephrine and dopamine are involved in the mediation of sexual receptivity.

# BACKGROUND

The periodic appearance of behavioral receptivity is dependent upon the cyclic secretion of estrogen and progesterone from the ovary. In the cycling female rat, each cycle is 4-5 days in length, with the period of behavioral estrus lasting approximately 12 hours. The onset of sexual receptivity is synchronized with the release of mature ova from the ovary. Ovulation and the period of behavioral 'heat' follow two days of estrogen secretion and a pulse of progesterone from the ovary and adrenal gland on the eve of proestrus (Barraclough et al., 1973; Feder et al., 1968).

Following ovariectomy in rats, the estrous cycle and receptivity are immediately and permanently abolished (Ball, 1936; Beach, 1942). However, estrous behavior can be restored in ovariectomized rats by the daily administration of estrogen (Davidson et al., 1968). This has also been established in the hamster (Frank & Fraps, 1945), cat (Bard, 1939), dog (Robson, 1938) and monkey (Ball, 1936).

The ovariectomized rat usually exhibits low levels of sexual receptivity following estrogen-administration alone, but this response is intensified by a single injection of progesterone (e.g., Beach, 1942; Dempsey et al., 1936). To artificially induce estrus, estradiol benzoate (EB), 3-10 μg/animal, is usually injected 48 hr prior to a single injection of progesterone (500 μg/animal). Four

to six hr after progesterone administration the female will readily copulate with the male.

In the following two sections a description of female as well as male sexual behavior will be presented.

## Female Sexual Behavior

The copulatory pattern of the normal female rat consists of two major components: (1) proceptive behaviors (i.e., hopping and darting); and (2) the lordosis reflex. The main feature of proceptive behavior are hopping and darting, which are staccato-like alternations between running and stopping, often accompanied by rapid ear-wiggling. This component is often exhibited by the highly receptive female rat. The lordosis-reflex consists of an anti-dorsiflexion of the back, extension of the neck and a lateral deviation of the tail. The adoption of this posture results in the exposure of the female's perineum, which facilitates penetration of the vaginal orifice (an intromission) by the mounting male.

## Male Sexual Behavior

The masculine pattern of sexual behavior consists of three principal components; mounts, intromissions, and ejaculations. Following the introduction of the female into the testing arena, the female is investigated by the male. Following mutual investigatory behavior, the male will mount the female from the rear, palpating her flanks with his forelegs. While clasping the female in this manner the male may thrust his pelvic region several times (mounts with thrust). Following this behavior the male dismounts and in a

short while (30-60 seconds) mounts the female again. If the dismount is weak or passive this usually indicates the lack of an intromission (penile insertion). An intromission is characterized by a series of rapid thrusts followed by a single deep thrust; a rapid kick with a single hindleg culminating with a rapid backward withdrawal from the female (Young, 1961). After a series of intromissions an ejaculation will occur. During an ejaculation, immediately preceding the posterior withdrawal, the male will move his front paws laterally while moving to an upright position, and at this time the ejaculate is expelled. After an ejaculation there is usually a 4-8 minute period with no copulatory behavior.

## Neural Control of Adult Female Sexual Behavior

### Lesion Studies

The results of several studies suggest that the diencephalon is critical for the mediation of female sexual behavior. Early lesion studies by Bard (1939) suggested that the hypothalamus is critical for the occurrence of the lordosis reflex. Law et al. (1958), Singer (1968), Kennedy (1964), and Herndon and Neil (1973) have reported that lesions restricted to the anterior hypothalamus decrease or abolish female sexual behavior.

On the other hand, restricted lesions of the MPOA (Dorner et al., 1969; Powers & Valenstein, 1972); the lateral septum (Nance et al., 1974, 1975a, 1975b) and the olfactory bulb (Nance et al., 1976) have been reported to facilitate lordotic behavior in the estrogen-primed female rat. The effect of the lesions produced

in the latter group of experiments has been interpreted as resulting in the destruction of cells within a tonic inhibitory system of neurons, thus resulting in the facilitation of female sexual behavior. Since none of these lesions resulted in the facilitation of sexual receptivity in the absence of exogenous estrogen, the effect of these lesions may have been to increase the animal's behavioral sensitivity to, and/or decrease the rate of metabolism of, estrogen (Powers & Valenstein, 1972; Rodgers & Schwartz, 1976).

Neuroendocrine Control

Intracerebral treatment of the basal diencephalon, which includes an area from the anterior hypothalamus to the caudal mammillary region, with estrogen results in the enhancement of sexual receptivity in ovariectomized cats (Harris & Michael, 1958) and rabbits (Palka & Sawyer, 1966a). However, the estrogen-sensitive mechanisms within the diencephalon of the female rat exist in a more circumscribed area. Estrogen implanted in the MPOA-AH or the ventral medial hypothalamus of the female rat facilitates sexual receptivity, whereas implants slightly posterior have no effect (Dorner et al., 1969; Lisk, 1962).

The locus of progesterone's central action in the female rat is probably within the midbrain. Following ovariectomy and estrogen-priming, females treated with progesterone in the midbrain (dorsal to the interpeduncular nucleus) showed a dramatic increase in lordotic behavior, while progesterone applied to the MPOA-AH, lateral POA, and to the anterior hypothalamus was without effect

(Ross et al., 1971). That the effective site of action of progesterone is within the midbrain has received support from studies in which the uptake and retention of progesterone was observed. It was concluded from these reports that progesterone is concentrated most actively by cells within the midbrain (Whalen & Luttege, 1971; Wade et al., 1973).

In summary, sexual behavior in the female rat is under the control of the ovarian hormones estrogen and progesterone. The influence of estrogen and progesterone upon sexual receptivity has been shown to involve separate brain sites; and may involve a number of different neuro-pharmacological processes.

## Neuro-pharmacological Control

### Cholinergic Systems

There is evidence that acetylcholine (ACh) acts as a transmitter in the central nervous system, and that its action is predominantly excitatory (Karczmar, 1971). Choline acetyltransferase (ChAC) is the enzyme which is responsible for the synthesis of ACh, while acetylcholinesterase (AChE) is responsible for its catabolism. These enzymes are used as histochemical markers for ACh, and have been identified throughout the central nervous system (Lewis & Shute, 1967; Shute & Lewis, 1967; McGeer et al., 1974) and within specific limbic system nuclei (Palkovits et al., 1974; Brownstein et al., 1975, 1976; Fonnum et al., 1977). These recent biochemical data indicate that the estrogen-sensitive MPOA-AH and the progestin-sensitive midbrain are heterogeneous; for example, choline

acetyltransferase is present throughout the anterior and posterior hypothalamic nuclei (Brownstein et al., 1975), however, the highest concentration of choline acetyltransferase is found near the interpeduncular nucleus in the MRF (Lewis & Shute, 1967; Kataoka et al., 1973; Palkovits et al., 1974; Fonnum et al., 1977). Thus, when the biochemical data are compared to those of histochemical studies, it is apparent that the anterior hypothalamus and midbrain structures, which are known to be responsive to ovarian hormones, are also rich in ACh and its synthesizing enzymes.

The presence of ACh may not correlate with the presence of acetylcholinesterase (Jacobowitz & Goldberg, 1977). The medial preoptic area and the anterior hypothalamic nuclei contain only a sparse number of AChE-containing fibers, although these nuclei were observed to contain high concentrations of ACh (Jacobowitz & Goldberg, 1977). On the other hand, while ACh concentrations have not been determined in separate areas within the midbrain, intense AChE staining of the neuropil occurs near the interpeduncular nucleus (Palkovits & Jacobowitz, 1974; Fonnum et al., 1977).

It has been proposed that two types of cholinergic receptors exist in the central nervous system (Schleiffer & Eldefrawi, 1974; Schlecter & Rosecrans, 1971). Recent studies using labelled alpha-bungarotoxin (Eterovic & Bennet, 1974; Polz-Tejera et al., 1975) and using muscarinic antagonists (Polz-Tejera et al., 1975; Yamamura et al., 1974) offer direct evidence for the existence of nicotinic and muscarinic receptors in the brain. It has been suggested that the majority of synaptic receptors in the midbrain are muscarinic

in nature (Lake, 1973; Kuhar et al., 1975; Hattori et al., 1977). Nicotinic receptors have also been identified within the midbrain and hypothalamus (Morley et al., 1977).

Choline acetyltransferase, in addition to being present in the estrogen-sensitive MPOA-AH, has been shown to be responsive to exogenously administered hormone treatment (Luine et al., 1975) and to cyclic variations during the estrous cycle (Kobayashi et al., 1966). Exogenously administered EB was observed to increase ChAc levels in the MPOA-AH of ovariectomized female rats.

These data taken together are conducive to the idea that estrogenic induction of sexual receptivity may be mediated by cholinergic neurons located in the estrogen-sensitive medial preoptic area.

Systemic administration of cholinergic compounds has been reported to facilitate lordosis in the estrogen-primed female rat. More specifically, systemic administration of the muscarinic agonists pilocarpine and oxotremorine, or the nicotinic agonist nicotine, significantly facilitated lordotic behavior when given after estrogen-priming (Fuxe et al., 1977; Lindstrom, 1973).

Following administration of muscarinic agonists, facilitation of lordosis was not observed until at least 3 hr after injection of the compound. This time-delay offers support for the suggestion that the pituitary-adrenal axis (and subsequent progesterone release) may be participating in the facilitation of sexual receptivity. This suggestion was supported by the results of other experiments which demonstrated that adrenalectomy or hypophysectomy

abolished the activation of lordotic behavior following systemic treatment with muscarinic agonists (Lindstrom, 1973).

The mechanism of action of systemically administered nicotine on female sexual behavior is as yet unknown. That the facilitative effects of nicotinic stimulation were due to indirect activation of the pituitary-adrenal axis and release of progesterone is opposed by the short time-course of action for the drug. Systemic administration of nicotine was observed to increase lordosis frequency within 5 min (Fuxe et al., 1977), while adrenal progesterone does not activate lordosis until at least 4 hr after its release (Feder & Ruf, 1969). These data are in contrast to the facilitative effects of muscarinic agonists on lordosis in estrogen-primed female rats, which are clearly related to the release of adrenal progestins (Lindstrom, 1973).

These data (Fuxe et al., 1977; Lindstrom, 1973) do not provide unequivocal support for the suggestion that systemic cholinergic manipulations mediate sexual receptivity. The failure of systemic cholinergic manipulations to facilitate receptivity in estrogen-primed, adrenalectomized rats is consistent with the hypothesis that the facilitative effects may be mediated by the pituitary-adrenal axis.

## Serotonergic Systems

Serotonin (5-hydroxytrptamine) is unevenly distributed in the rat brain (Bogdanski et al., 1957; Passonen et al., 1975); the highest concentrations are found in the hypothalamus and the

brainstem. The raphé nuclei (cell groups B7 and B8) in the brainstem, contain the largest number of serotonin-containing cell bodies (Dahlstrom & Fuxe, 1964; Ungerstedt, 1971). Axons from the B 7, B 8, and B 9 cells ascend in the medial forebrain bundle (MFB) to innervate many forebrain regions (Dahlstrom & Fuxe, 1964; Fuxe, 1965; Ungerstedt, 1971). The results of more detailed studies of central 5-HT pathways suggest that three distinct ascending 5-HT tracts may be dileneated (Fuxe & Jonsson, 1973). The first, a medial subcortical 5-HT pathway arises mainly from B 8 cells in the median raphé. These cells give rise to axons which course medial to the fasiculus retroflexus before ascending in the MFB lateral to the fornix. These axons innervate the hypothalamus and preoptic regions.

The second ascending 5-HT tract comprises a lateral mesencephalo-cortical 5-HT pathway (Fuxe & Jonsson, 1973). This pathway ascends from the B 7-9 cell groups (mainly B 7) and also ascends in the MFB. These axons innervate the dorsal cortex, hippocampus, septum, olfactory tubercle and amygdala.

The third ascending 5-HT pathway forms a neostriatal 5-HT pathway that ascends from B 7, B 8, and B 9 cells, on the medial surface of the crus cerebri. These axons provide a sparse distribution of serotonergic terminals scattered in the caudate putamen (Fuxe & Jonsson, 1973).

The presence of serotonin has been demonstrated within the medial nuclei of the hypothalamus of the rat (Fuxe, 1965; Saavedra et al., 1974). High serotonin content was found in the suprachiasmatic and the arcuate nuclei, the preoptic area, and the

posterior hypothalamus. Likewise, enzymes involved in the biosynthesis of serotonin, e.g., tryptophan hydroxylase, have also been detected in these hypothalamic areas (Saavedra, 1974).

The high concentrations of serotonin in nuclei associated with the action of estrogen and progesterone offer support for the suggestion that serotonin may participate in the regulation of behavioral receptivity.

A large volume of psychopharmacological studies suggests that sexual behavior in the estrogen-primed female rat is mediated by a 5-HT neural system (Meyerson, 1964a, b, c,; 1966a, b; 1968). This hypothesis is usually supported by two lines of evidence: (1) that suppression of ongoing sexual receptivity occurs after activiation of 5-HT receptors (Meyerson, 1964), potentiation of 5-HT release (Everitt et al., 1975a; Zemlen et al., 1977) or elevation of 5-HT levels (Espino et al., 1975; Meyerson, 1964a); and (2) that disinhibition of female sexual behavior occurs following the depletion of 5-HT (Everitt et al., 1975a; Zemlen et al., 1973).

While there is a considerable body of evidence which supports the hypothesis that serotonin mediates synaptic transmission between cells which inhibit female sexual behavior, there are several studies which make this serotonergic hypothesis suspect. For example, alpha-propyldopacetamide, which is as effective as PCPA (parachlorophenylalanine) in reducing brain 5-HT levels, was ineffective in facilitating estrous behavior in estrogen-primed, ovariectomized female rats (Meyerson & Lewander, 1970). Secondly, reserpine and PCPA failed to facilitate sexual receptivity in adrenalectomized

guinea pigs (Paris et al., 1971), rats (Eriksson & Sodersten, 1973), and mice (Hansult et al., 1972; Uphouse et al., 1970). Thus, administration of these compounds may facilitate sexual receptivity in non-adrenalectomized animals by facilitating the secretion of adrenal progesterone, which in turn stimulates sexual behavior.

In contrast to these findings, it has been reported that PCPA does facilitate sexual receptivity in estrogen-primed, ovariectomized, adrenalectomized female rats (Everitt et al., 1975a; Zemlen et al., 1973). The problems surrounding the interpretation of these systemic pharmacological studies are compounded by the indirect actions of the drugs used, time intervals following drug administration and behavioral testing, methods of testing for sexual receptivity, and the criteria of evaluating the lordosis reflex. Furthermore, these studies illustrate that adrenalectomized animals may represent a pathological preparation; that is, the failure of systemically administered compounds to facilitate sexual receptivity may be due to the animal's deteriorating health. Thus, for the reasons outlined above the results of systemic pharmacological studies must be interpreted with caution.

Perhaps more localized modifications of serotonergic systems might give a better indication of the influence of specific brain systems on female sexual behavior. Direct intracerebral treatment in the anterior hypothalamus or along the medial forebrain bundle with the serotonergic antagonists, cinanserin and methysergide, increased the probability of lordosis within 30 min (Ward et al., 1975). These data suggest that localized antagonism of

serotonergic receptors in the estrogen-sensitive anterior hypothalamus facilitates sexual receptivity. That the adrenal gland is not involved in the mediation of these effects is suggested by the observation that intrahypothalamic application of methysergide or cinanserin, enhanced lordosis frequency in male rats. Male rats are normally not responsive to the synergistic action of estrogen and progesterone (Clemens et al., 1970; Davidson & Levine, 1969).

Increases in lordotic responding have also been observed following direct central modification of serotonergic activity; e.g., depletion of 5-HT following intracerebral injection of the neurotoxin 5,7-dihydroxytryptamine enhances lordosis frequency (Everitt et al., 1975b). When 5,7-dihydroxytryptamine was injected into the medial ascending 5-HT pathways, the uptake of ($^{3}$H) 5-HT was reduced; furthermore, the time course of the behavioral effect followed the reduction of 5-HT in the hypothalamus.

These data suggest that facilitation of sexual receptivity is possible following direct central antagonism of serotonergic receptors, and offer support for the suggestion that these facilitative effects are confounded or masked following systemic administration of similar compounds.

In addition to being estrogen-sensitive, and having both cholinergic and serotonergic profiles, the MPOA is also a site of convergence of noradrenergic, dopaminergic and adrenergic fiber systems which ascend from their cell bodies of origin within the brainstem.

Catecholaminergic Systems

The ventral noradrenergic (NA) pathway has cell bodies of origin in the A1, A2, and A7 cell groups in the medulla oblongata and the pons. Axons of these cells ascend within the medial forebrain bundle to innervate the mesencephalon, medial preoptic and anterior hypothalamus. Cell groups A8, 9, and 10 in the ventral midbrain contain DA neurons which give rise to ascending DA systems innervating the forebrain. The nigrostriatal DA system arises from cells in the A8-9 groups within the zona compacta of the substantia nigra and projects to the caudate-putamen (Ungerstedt, 1971). A second system, the mesolimbic DA system, originates in the A10 cell group which is located dorsal to the interpeduncular nucleus. Axons from these cells ascend dorsally in the MFB and innervate the nucleus accumbens, dorsal part of the nucleus interstial stria terminalis, and the olfactory tubercle. A third DA pathway, the tuberoinfundibular DA system, is found in the mediobasal hypothalamus. This system originates from group A12 DA neurons; its cell bodies are located in acruate nuclei, and periventricular nuclei at the level of the ventromedial hypothalamus. These cells give rise to fibers which project ventrally to the median eminance.

The enzymes responsible for the synthesis of catecholamines are unevenly distributed within the hypothalamus. Tyrosine hydroxylase, dopamine-β-hydroxylase, and phenethylanolamine N-methyltransferase are all present in the basal hypothalamus and the medial hypothalamic nuclei (Saavedra, 1974).

There is considerable evidence upon which to suggest that ovarian hormones modulate the activity of these neurotransmitters. For example, chronic administration of estrogen and progesterone decreases brain NE and DA (Greengrass & Tonge, 1972); while progesterone administration alone increases NE turnover in the whole brain (Hackman et al., 1973). During estrus there is a marked increase in DA (Zschack & Wurtman, 1973). On the other hand, ovariectomy increases NE synthesis within the anterior hypothalamus; this effect may be reversed by estrogen administration (Bapna et al., 1971). No change in amine metabolism was observed in the posterior hypothalamus (Bapna et al., 1971).

The enzymes which synthesize and degrade biogenic amines are also sensitive to alterations in steroid hormone levels. Hypothalamic monoamine oxidase (MAO) has been shown to fluctuate through the estrous cycle (Holzbauer & Youdim, 1973; Kamberi & Kobayashi, 1970; Kobayashi et al., 1966). More recently, it has been demonstrated that estrogen-pretreatment increased MPOA levels of MAO, the enzyme responsible for the deactivation of catecholamines (Luine et al., 1975). Although much of the research concerning the role of monoamines in regulating female reproductive behavior has concentrated on serotonin, several recent reports have suggested that catecholamines may also be involved. For example, systemic administration of compounds which increases noradrenergic, or reduces dopaminergic or adrenergic neurotransmission, has been reported to facilitate lordosis in the estrogen-primed female rat (Ahlenius et al., 1972a; Everitt et al., 1974; Everitt et al.,

1975a, b). In addition, systemic administration of alpha-adrenergic agonists (clonidine) has been reported to be without facilitative effects (Davis & Kohl, 1977), while systemic administration of alpha-adrenergic antagonists (yohimbine or phenoxybenzamine) resulted in an increased probability of lordotic behavior in the estrogen-primed female rat (Everitt et al., 1975a, b). However, the non-specific modification of all adrenergic and noradrenergic brain systems achieved by systemic drug administration may obscure the role(s) of these transmitters within specific brain systems such as the MPOA. In addition, the difficulty in interpreting the results of systemic pharmacological treatments lies in the failure to take into account the spatial organization of the brain; thus, these studies are severely limited in terms of their usefulness in localizing and identifying the specific areas of the brain involved in the regulation of female sexual behavior.

In an attempt to localize specific sites of action the beta-adrenergic blocker LB-46 was administered intrahypothalamically. It was observed that central administration of the adrenergic blocking compound LB-46 facilitated lordosis (Ward et al., 1975). It was hypothesized that beta-adrenergic blockage may facilitate occupation of central alpha-adrenergic receptors which elicit lordotic behavior (Ward et al., 1975).

## OBJECTIVES OF THE PRESENT STUDIES

The experiments in this dissertation were divided into three parts (Parts A, B, and C).

Part A. Cholinergic Brain Mechanisms and the Regulation of Sexual Behavior in the Female Rat. Experiment 1: The objective of this experiment was to determine if the mesencephalic reticular formation (MRF) is a site where direct administration of cholinergic drugs facilitate sexual behavior. Data from this experiment provided information on the facilitative effects of direct cholinergic stimulation on lordosis. Experiment 2: The objective of this experiment was to determine whether the posterior hypothalamus was a site of cholinergic action. Data from the present experiment provided information on the sites non-responsive to cholinergic stimulation. Experiment 3: The objective of this experiment was to study the contributions of muscarinic and nicotinic receptor systems within the MPOA and the MRF in the regulation of lordosis. It was observed that cholinergic systems within the brain differentially influence female sexual behavior. Experiment 4: The role of the adrenal secretions in mediating female sexual behavior was investigated in this experiment. This experiment demonstrated that following adrenalectomy the facilitative effects of cholinergic brain stimulation were still observed.

Part B. Central Serotonergic Systems; Possible Interactions with Central Cholinergic systems, and Female Sexual Behavior. Experiment 1: The objective of the present experiment was to determine the influence of serotonergic blockade in the medial preoptic-anterior hypothalamus (MPOA-AH) on female sexual behavior. Direct administration of serotonergic receptor antagonists into the MPOA-AH has previously been reported to facilitate lordosis in estrogen-primed female rats (Ward et al., 1975). This experiment was designed to replicate these findings. Results of the experiment provided information relevant to the question of whether antagonism of serotonergic processes within the MPOA facilitates sexual receptivity. Experiment 2: The objective of the present experiment was to determine the influence of enhanced serotonergic neurotransmission or serotonergic blockade within the MPOA on hormone-activated sexual behavior. Data on the effects of substances which antagonize (methysergide and cinanserin) or facilitate serotonergic neurotransmission (serotonin; 5-HT) were collected in tests with animals which had been treated with estrogen and progesterone. Experiment 3: The objective of the present experiment was to determine the influence of sequential serotonergic/cholinergic blockade on female sexual behavior. The data from this experiment provided information concerning the interactions between serotonergic and cholinergic systems in the facilitation of sexual receptivity.

Part C. Influence of Catecholamines in Mediating Female Sexual Behavior. Experiment 1: The objectives of the present experiment was to ascertain the contributions of alpha- and

beta-adrenergic systems within the MPOA in the regulation of lordosis. The data from the present study provided information on the role of alpha- and beta-noradrenergic receptors on sexual behavior in the female rat. Experiment 2: The objectives of the present experiment was to determine the influence of dopaminergic and noradrenergic systems in mediating hormone-activated sexual behavior. The data from the present investigation provided information on the relationship of catecholamines (e.g., NE and DA) within the MPOA to female sexual behavior.

# PART A. CHOLINERGIC BRAIN MECHANISMS AND THE REGULATION OF SEXUAL BEHAVIOR IN THE FEMALE RAT

The present study was designed to permit an evaluation of the effects of central cholinergic stimulation on female sexual behavior. Sites of cholinergic action were located; non-responsive control sites were identified; contributions of nicotinic and muscarinic systems were ascertained; and, additionally, the possible role of the adrenal gland in mediating the facilitative effects of cholinergic stimulation was examined.

## General Methods

Details concerning animals, surgical and hormonal treatments, cannulation procedures, behavioral observation and testing procedures, and chemical stimulation techniques are described in Appendices A, B-1 and B-2, C, and D (pages 92-99).

Briefly, experimental females were selected on the basis of their ability to show sexual responses to suprathreshold doses of estradiol benzoate (1 μg/animal/day, for three days) and progesterone (0.5 mg/animal. To make this selection, ovariectomized females were tested for lordosis prior to intracerebral implantation. One week after ovariectomy, females were treated with estradiol benzoate (EB, 1 μg/animal/day, for three days, at approximately 0800-0900 hr),

followed by 0.5 mg progesterone (P) on the fourth day (at approximately 900-1000 hr). Four to six hours after the progesterone injection each animal was given a test for sexual receptivity. Nearly all females are receptive following the above hormone treatment; those females which were not receptive were not used in the following experiments.

A sexual receptivity test consisted of placing the female in a cage with a sexually active Long-Evans male rat until the male mounted the female 10 times. A receptive female will normally show a dorsal concave arching of the back (lordosis) when mounted by the male. Lordosis frequency of the female in response to mounts by the male was used as a measure of sexual receptivity. This measure was expressed as lordosis quotient (LQ: Lordosis Frequency/10 mounts x 100). In addition, soliciting behaviors (e.g., hopping, darting, and ear-wiggling) by the female were noted. Female rats which achieved an LQ of 50 or greater were retained for intracerebral implantation.

To administer cholinergic compounds into the brain, stainless steel cannulae were unilaterally (Experiments 1 and 2), or bilaterally (Experiments 3 and 4) implanted into four groups of ovariectomized or ovariectomized/adrenalectomized Sherman strain female rats (see Appendixes A and B). This double-cannula system, consisting of two concentrically mounted stainless steel cannulae, allows for the deposition of minute amounts of cystalline chemicals at the selected site by packing the chemicals into the tip of the removable inner cannula, and the reinserting it into the implanted

guide cannula (Grossman, 1960). Animals were implanted, under light ether anesthesia, eleven days prior to experimental behavior testing.

Beginning one week after implantation all groups of ovariectomized females were injected intramuscularly with 1 µg of EB in sesame oil for three days prior to testing (see Appendix C). On the fourth day, all females were given a 125 µg injection of dexamethasone to reduce adrenal activation (Paris et al., 1971), and a test for sexual receptivity four hr later. Although the dosage of EB used in these experiments does not normally induce high levels of sexual activity without progesterone, all females were given a 10 mount-pretest immediately prior to intracerebral treatment, and those females which achieved an LQ of 30 or greater on this pretest were considered receptive and eliminated from further testing. The remaining females were treated intracerebrally and tested 30, 60, and 120 min later.

Following the pretest the inner cannulae were removed and loaded by tapping them five times into a thin layer of a crystalline compound spread on a glass plate; these inner cannulae were then returned to the permanently implanted guide cannulae. This removal and reinsertion of the inner cannulae was accomplished while the rats were unanesthetized, and with little apparent stress to the rats.

At the completion of testing each inner cannula was removed and visually inspected under a dissecting microscope. If any chemical remained in the lumen, the female was eliminated from the

experiment. Any female demonstrating adverse drug effects during the behavioral testing was eliminated from further testing.

At the conclusion of each of the experiments in Part A, the females were perfused through the heart with saline and 10% formalin solution (see Appendix E); the brains were excised, embedded in paraffin, and serially sectioned at 30 μ. All sections were then stained with luxol fast blue for fibers and counterstained with cresyl violet for cell bodies, and examined for confirmation of the implantation sites.

## Experiment 1. Sites of Cholinergic Action: The Midbrain Reticular Formation

### Methods

To determine whether cholinergic stimulation of the progesterone-sensitive mesencephalic reticular formation (MRF) would influence lordosis, 11 females were implanted unilaterally with cannulae in the MRF. Coordinates for the implants were taken from the atlas of Albe-Fessard et al. (1966), and were: AP 1.4 Lat. 1.0, and Vert. 2.75.

Each female was tested on two consecutive weeks; in each test the rats were treated intracerebrally with 10-15 μg of carbamylcholine chloride (Carbachol; Sigma) and tested 30, 60 and 120 min later. Sixty min before intracerebral treatment, half of the female rats were given intraperitoneal injections of the cholinergic antagonist, atropine sulfate (2.5 mg/animal, in .1 cc of saline; Sigma); the remaining half were given an equal volume

of saline. One week later animals receiving saline were given atropine; at this time animals which received atropine were given a saline injection.

Results

Unilateral cholinergic stimulation of the MRF resulted in a facilitation of lordosis in 10 of 11 animals. Table 1 shows the mean LQs at the pretest (PT) and on the repeated tests following intracerebral treatment with the cholinomimetic, carbachol. None of the animals achieved a lordosis during the 10 mount-pretest preceding intracerebral treatment. A significant increase in lordosis was observed 30, 60, and 120 min after intracerebral treatment with carbachol ($p < .001$; Pretest (PT) versus scores from the 30, 60, and 120 min-tests; see Appendix F for details concerning statistical analysis of the data from Part A). When the animals were pretreated with the cholinergic antagonist, atropine sulfate (2.5 mg/animal), the animals failed to achieve statistically significant levels of lordosis in any of the post-intracerebral tests ($p < .05$; grouped scores from saline test versus scores from the atropine sulfate tests).

Figure 1 shows the histological placement of the lordosis-positive and lordosis-negative implant sites. The ten positive sites for cholinergic implants were caudal and immediately dorsal to the interpeduncular nucleus; the non-responsive implant site was more ventral and caudal.

TABLE 1.--Mean lordosis quotients of estradiol benzoate (EB)-primed female rats following intrareticular treatment with carbachol in the mesencephalic reticular formation (MRF). All values are means ± SEM.

| Treatment[a] | | Post-treatment Time in Minutes | | | | |
|---|---|---|---|---|---|---|
| | N[b] | Pretest | 30 | 60 | 120 | P |
| Carbachol & Saline | 11 | 0.0 | 71.0 ± 7.8** | 71.7 ± 7.8** | 64.0 ± 8.4** | **=P < .001 |
| Carbachol & Atropine Sulfate | 11 | 0.0 | 6.0 ± 3.3* | 9.0 ± 2.7* | 16.0 ± 9.5* | *=P < .05 |

[a]Treatment; carbachol (10-15 μg, intrareticular); Atropine Sulfate (2.5 mg/animal, i.p.).

[b]Includes both lordosis-positive and lordosis-negative animals (n=1).

*Scores from the atropine sulfate test scores versus saline test scores.

**Pretest scores versus scores during 30, 60, and 120 min. tests.

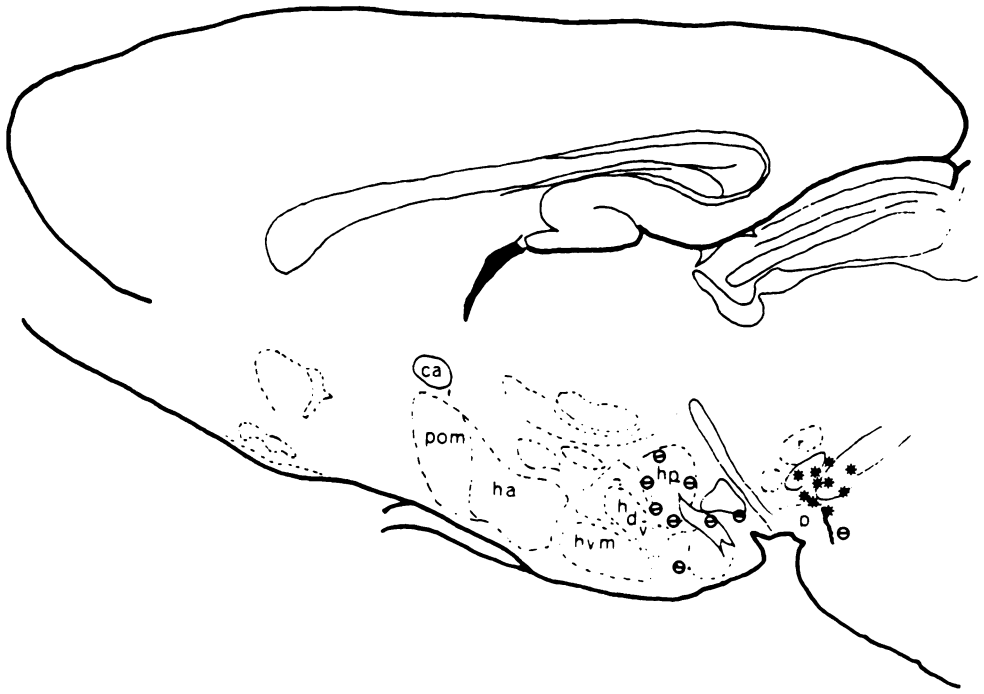



Figure 1.--Parasagittal view (1 mm lateral) of diencephalon and mesencephalon showing sites of carbachol, progesterone, and cholesterol implants in ovariectomized EB-primed female rats. Lordosis-positive loci are indicated by the following symbol (*). Lordosis-negative loci are indicated by the following symbol (⊖). Lordosis-positive = LQ of 30 or greater on any post-intracerebral treatment test; Lordosis-negative = LQ of 0-20 on any post-intracerebral treatment test. Implant loci indicated by stars (*) are sites where carbachol treatment facilitated sexual behavior; lordosis-negative sites (⊖) within the PHA are sites where carbachol, progesterone, or cholesterol failed to affect lordotic behavior. Abbreviations: anterior commissure (ca), medial preoptic area (pom), anterior hypothalamus (ha), ventral medial hypothalamus (hvm), dorsal portions of ventral hypothalamus (hdv), posterior hypothalamus (hp), red nucleus (r), interpeduncular nucleus (ip).

## Experiment 2: Sites of Cholinergic Action: The Hypothalamus

### Methods

To determine whether direct administration of the cholinergic compound, carbachol, and/or progesterone would be effective in activating lordotic behavior, eight additional females were implanted unilaterally with cannulae at another progesterone-sensitive brain locus: the posterior hypothalamus (PHA). Coordinates for the implants were taken from the atlas of Albe-Fessard et al. (1966), and were: AP 4.5, Lat. 1.0, and Vert. 3.0.

Estrogen-primed female rats were tested weekly over three consecutive weeks until each animal had been tested with carbachol (10-15 µg); progesterone (15-25 µg), or cholesterol (15-25 µg). The order of experimental testing for these three conditions was randomized for each female.

### Results

Unilateral stimulation of the PHA with carbachol, progesterone, or cholesterol failed to facilitate lordosis ($p > .05$). Table 2 shows the mean LQs on the pretest (PT) and on repeated tests following intracerebral treatment.

Figure 1 shows the histological placement of these lordosis negative implant sites, which were all found to lie within the PHA.

TABLE 2.--Mean LQ of EB-primed female rats following intrahypothalamic treatment with carbachol, progesterone, or cholesterol. All values are means ± SEM.

| Treatment | N | Post-treatment Time in Minutes | | | |
|---|---|---|---|---|---|
| | | Pretest | 30 | 60 | 120 |
| Carbachol (10-15 µg) | 8 | 0.0 | 3.7 ± 2.6 | 7.5 ± 5.2 | 15.0 ± 12.4 |
| Progesterone (15-25 µg) | 8 | 0.0 | 0.0 | 0.0 | 0.0 |
| Cholesterol (15-25 µg) | 8 | 0.0 | 0.0 | 0.0 | 0.0 |

## Experiment 3. Contributions of Nicotinic and Muscarinic Systems Within the MRF and the MPOA

### Muscarinic Systems

Methods.--The results of several experiments suggest that the use of carbachol as a cholinomimetic may be potentially misleading. For example, application of carbachol into the MRF produced; (1) in addition of cholinergic effects, a non-specific action on non-cholinoceptive neurons (Kent, 1972); (2) motor seizures (Routtenberg, 1965), and (3) sensory hyperactivity (Grossman, 1968).

In Experiment 1 it was observed that lordotic behavior was induced by intrareticular administration of carbachol into the MRF. Becuase carbachol retains a high level of both nicotinic as well as muscarinic activity (Goodman & Gilman, 1970), Experiment 3 was designed to determine whether this central cholinergic effect is based on muscarinic or nicotinic receptor activity. Thus, to determine whether muscarinic stimulation of the MRF and the estrogen-sensitive MPOA would influence lordosis, eleven ovariectomized female rats, selected on the basis of their response to estrogen and progesterone (General Methods and Appendix B-1) were bilaterally implanted with cannulae (General Methods and Appendix B-2). Six female rats were implanted in the MRF; coordinates were identical to those given in Experiment 1. Five female rats were implanted in the MPOA; coordinates were taken from the atlas of Albe-Fessard et al. (1966), and were: AP 8.2, Lat. 1.0, and Vert. 3.5.

After implantation each female was tested once per week for two consecutive weeks. On each test females were given the standard

estrogen, dexamethasone-pretreatment followed by intracerebral administration of the muscarinic agonist carbamylmethylcholine chloride (10-15 µg/side, bethanechol; Sigma). On the first test, half of the animals in each group received a systemic injection of atropine sulfate sixty minutes prior to pretesting, and the other half received saline. On the following systemic injections of saline and atropine were reversed. Immediately prior to intracerebral treatment each animal was given a 10 mount-pretest. After intracerebral treatment each animal was then tested 30, 60, and 120 min later.

Results.--Stimulation of the MRF or the MPOA with the muscarinic agonist bethanechol resulted in a significant increase in lordotic behavior. Following stimulation of the MRF, a significant increase in lordotic behavior was observed on all three post-treatment tests (Table 3; $p < .05$; Pretest (PT) versus scores from the 30, 60, and 120 min tests). However, following stimulation of the MPOA a significant increase in lordotic behavior was observed only on the 60 and 120 min-tests ($p < .001$; Pretest (PT) versus scores from the 60 and 120 min-tests). Systemic pretreatment with the muscarinic antagonist, atropine sulfate, inhibited the muscarinic-induced facilitation in both the MRF and the MPOA Table 3).

Histological examination of the MPOA implants revealed that the four lordosis-positive, and one lordosis-negative site(s), were located in the MPOA (Figures 2 and 3). MRF implants were found

TABLE 3.--Mean LQs of EB-primed female rats following intrareticular (MRF) and intrahypothalamic (MPOA) treatment with bethanechol. All values are means ± SEM.

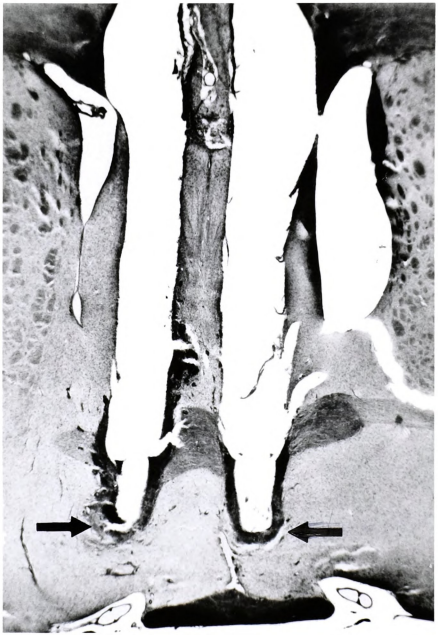
| Treatment[a] | N[b] | Post-treatment Time in Minutes | | | | |
|---|---|---|---|---|---|---|
| | | Pretest | 30 | 60 | 120 | P |
| MRF | | | | | | |
| Bethanechol (10-15 µg) & Saline | 6 | 5.0 ± 3.4 | 48.0 ± 14.0** | 60.0 ± 8.1** | 81.0 ± 7.0** | **=P < .001 |
| Bethanechol (10-15 µg) & Atropine Sulfate | 6 | 3.3 ± 2.0 | 5.0 ± 2.2 | 5.0 ± 2.2 | 6.6 ± 2.0 | |
| MPOA | | | | | | |
| Bethanechol (10-15 µg) & Saline | 5 | 0.0 | 5.0 ± 2.8 | 62.5 ± 18.8** | 100.0** | **=P < .001 |
| Bethanechol (10-15 µg) & Atropine Sulfate | 5 | 0.0 | 0.0 | 0.0 | 0.0 | |

[a]Treatments: Bethanechol (intracerebral); saline (.1 cc; systemic); Atropine Sulfate (2.5 mg/animal; systemic).

[b]Includes both lordosis-positive and lordosis-negative animals.

**Pretest scores versus 30, 60, and 120 min. test scores.

Figure 2.--Photomicrograph of frontal sections of the rat brain showing representative injection sites (indicated by arrows) for animals with bilateral cannulae aimed at the medial preoptic area.



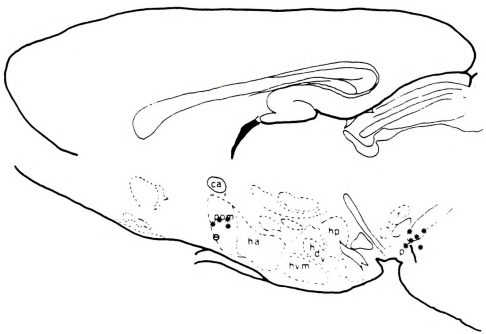



Figure 3.--Parasagittal view of the diencephalon and mesencephalon showing sites of muscarinic implants in EB-primed female rats. Lordosis-positive loci are indicated by the following symbol: (*). The lordosis-negative locus is indicated by the following symbol: (⊖). Lordosis-positive = LQ of 30 or greater on any of the post-intracerebral treatment tests. Lordosis-negative = LQ of 0-20 on any of the post-intracerebral treatment tests. Abbreviations are as those in Figure 1.

to lie immediately dorsal and caudal to the interpeduncular nucleus (Figure 3).

Nicotinic Systems

Methods.--To determine the influence of nicotinic stimulation in the MPOA and the MRF on female sexual behavior, an additional 11 female rats were bilaterally implanted, six in the MRF and five in the MPOA; coordinates were the same as those in Experiments 1 and 3. Each animal was tested once a week for two consecutive weeks following cannulation.

Females were treated with atropine sulfate (2.5 mg/animal, i.p.) one hour prior to each intracerebral treatment in order to eliminate muscarinic influence. Nicotinic receptor stimulation was achieved indirectly with bilaterally administration of 9-12 μg of neostigmine bromide (Sigma); intracerebral treatment with an anticholinesterase compound in an animal pretreated with atropine sulfate would presumably elevate ACh levels and, in the absence of muscarinic stimulation, would result in a nicotinic influence. Sixty minutes before intracerebral treatment half of the female rats were given an intraperitoneal injection of the nicotinic antagonist, mecamylamine HCL (2.5 mg/animal, in .1 cc saline); the remaining half were given an equal volume of saline. One week later the saline and mecamylamine treatments were reversed.

Results.--Administration of neostigmine into the MRF resulted in a significant increase in lordosis behavior on the 120 min-test

(Table 4; $p < .001$, Pretest (PT) versus 120 min-test scores). Systemic administration of mecamylalamine HCl significantly antagonized the facilitative effects of intracerebral neostigmine treatment upon lordosis (Table 4; $p < .01$; Scores from saline tests versus scores from mecamyalamine tests during the 120 min-test). Intracerebral administration of neostigmine within the MPOA, preceded by atropine, and saline or mecamylamine, was without significant behavioral effect (Table 4).

Histological examination revealed that three of the five lordosis-positive sites were dorsal to the interpeduncular nucleus (Figure 4); two other lordosis-positive implant sites encroached upon the dorsal border of this nucleus. A single lordosis-negative site was found to lie within the interpeduncular nucleus. Implants intended for the MPOA were found to lie within that nucleus.

## Experiment 4. Role of the Adrenal Gland in Mediating the Facilitative Effects of Cholinergic Stimulation

### Methods

As a control for the possibility that dexamethasone may not completely suppress adrenal secretions during intrahypothalamic stimulation, a separate group of female rats which had been ovariectomized and adrenalectomized was tested. Eight female rats were bilaterally implanted with cannulae in the MPOA. Coordinates for the implants were the same as those given in Experiment 3.

Each female was tested on two consecutive weeks; in each test the females were treated intrahypothalamically with bethanechol

TABLE 4.--Mean LQs of EB-primed female rats following intrareticular (MRF) and intrahypothalamic (MPOA) treatment of neostigmine bromide. All values are means ± SEM.

| | | Post-treatment Time in Minutes | | | | |
|---|---|---|---|---|---|---|
| | N[b] | Pretest | 30 | 60 | 120 | P |
| MRF | | | | | | |
| Neostigmine & Atropine Sulfate Saline | 6 | 0.0 | 8.3 ± 5.9 | 30.0 ± 15.8 | 64.0 ± 16.9** | ** = P< .001 |
| Neostigmine & Atropine Sulfate Mecamylamine | 6 | 0.0 | 10.0 ± 5.4 | 26.0 ± 6.0 | 8.0 ± 4.9* | * = P< .001 |
| MPOA | | | | | | |
| Neostigmine & Atropine Sulfate Saline | 5 | 0.0 | 0.0 | 0.0 | 0.0 | |
| Neostigmine & Atropine Sulfate Mecamylamine | 5 | 0.0 | 0.0 | 9.0 | 0.0 | |

[a]Treatments: Neostigmine (9-12 µg; intracerebral); saline (.1 cc; systemic); Atropine Sulfate (2.5 mg/animal; systemic); mecamylamine (2.5 mg/animal; systemic).

[b]Includes both lordosis-positive and lordosis-negative animals.

*Scores from saline treated group versus scores from mecamylamine tests.

**Scores from pretest versus 120 min. test.

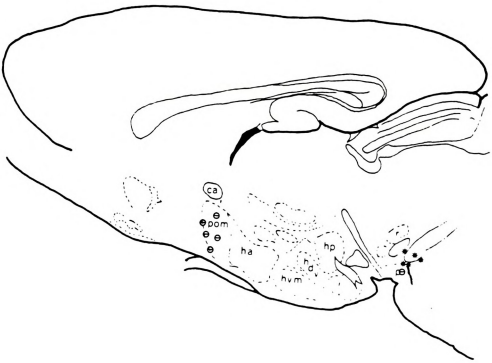



Figure 4.--Parasagittal view of the diencephalon and mesencephalon showing sites of nicotinic stimulation in EB-primed female rats. Lordosis-positive loci are indicated by the following symbol: (*). Lordosis-negative loci are indicated by the following symbol: (⊖). Lordosis-positive = LQ of 30 or greater on any post-intracerebral treatment test. Lordosis-negative = LQ of 0-20 on any post-intracerebral treatment test. Abbreviations are as in Figure 1.

(10-15 μg; muscarinic agonist), and tested 30, 60, and 120 min later. Sixty minutes prior to the intracerebral treatment half of the female rats were given an intraperitoneal injection of atropine sulfate (2.5 mg/animal); the remaining half of the females were given an equal volume of saline. One week later the saline and atropine treatments were reversed.

## Results

Bilateral administration of bethanechol into the MPOA facilitated lordosis in 7 of 8 adrenalectomized rats. Table 5 shows the mean LQs at the pretest (PT) and on the repeated tests. A significant increase in lordosis was observed 30, 60, and 120 min after the intrahypothalamic treatment and systemic injection of saline ($p < .001$; Pretest scores versus scores from the 30, 60, and 120 min-tests). Pretreatment with atropine sulfate antagonized this facilitative effect (Table 5; $p < .05$; Saline treated group scores versus atropine sulfate treated group scores, during the 30, 60, and 120 min-tests).

Histological examination (Figure 5) revealed that all eight implant sites were located within or encroaching upon the MPOA.

## Discussion

Intrahypothalamic or intrareticular administration of the cholinomimetic carbachol, as well as the anticholinesterase compound, neostigmine bromide, into the MPOA or the MRF increased the probability of lordosis in estrogen-primed female rats. Lordosis frequency was significantly increased by both muscarinic and

TABLE 5.--Mean LQs of EB-primed, adrenalectomized, female rats following intrahypothalamic (MPOA) treatment with bethanechol. All values are means ± SEM.

| Treatment[a] | N[b] | Post-treatment Time in Minutes | | | | P |
|---|---|---|---|---|---|---|
| | | Pretest | 30 | 60 | 120 | |
| Bethanechol (10-15 µg) & Saline | 8 | 4.2 ± 2.9 | 32.8 ± 8.6** | 54.2 ± 16.6** | 48.5 ± 13.0** | **=P < .001 |
| Bethanechol (10-15 µg) & Atropine Sulfate | 8 | 2.8 ± 2.8 | 2.5 ± 2.5* | 7.5 ± 4.9* | 13.7 ± 9.0* | *=P < .05 |

[a]Treatment: Bethanechol (intrahypothalamic), Atropine Sulfate (2.5 mg/animal, i.p.).

[b]Includes both lordosis-positive and lordosis-negative (n=1) animals.

**Pretest scores versus 30, 60, and 120 min. tests.

*Saline test scores versus the stropine sulfate test scores at the 30, 50, and 120 min tests.

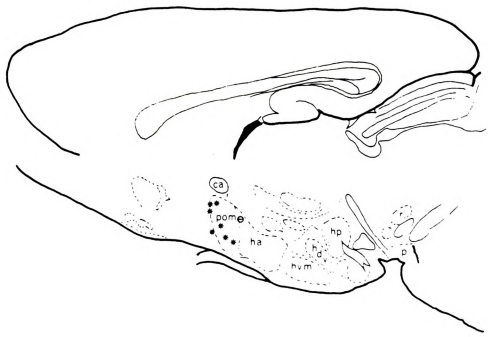



Figure 5.--Parasagittal view of the diencephalon and mesencephalon showing sites of muscarinic implants in ovariectomized/adrenalectomized, EB-primed female rats. Lordosis-positive loci are indicated by the following symbol (*). The lordosis-negative locus is indicated by the following symbol (⊖). Lordosis-positive = LQ of 30 or greater on any post-intracerebral treatment test. Lordosis-negative = LQ = 0-20 on any post-intracerebral treatment test. Abbreviations are as in Figure 1.

nicotinic receptor stimulation of the MRF; however, in the MPOA only muscarinic receptor stimulation facilitated sexual receptivity.

In addition, the inhibition of carbachol/bethanechol-induced facilitation of sexual receptivity by systemic administration of the cholinolytic compound, atropine sulfate (which readily penetrates the brain), supports the idea that the behavioral facilitation observed following cholinergic stimulation is due to the activity of central cholinergic systems.

Facilitation of sexual behavior following administration of cholinergic compounds may be attributed to diffusion of the drugs to adjacent neural areas. The finding that administration of carbachol to the progestin-sensitive PHA was ineffective in facilitating lordosis is, however, inconsistent with this interpretation. A possible explanation is that the PHA is less vascularized than the MPOA or the MRF. Thus, the cholinomimetics may have diffused from the MPOA and MRF and exerted an effect in a variety of subcortical regions.

Stimulation of the pituitary-adrenal axis is not a mechanism by which intracerebral treatment with bethanechol could facilitate sexual receptivity; this conclusion is supported by the observation that adrenalectomized females continued to show increased lordotic behavior in response to cholinergic stimulation.

It has previously been demonstrated that intrareticular treatment with progesterone in the MRF, dorsal to the interpeduncular nucleus, facilitated lordosis in the estrogen-primed female rat (Ross et al., 1971). This area also concentrates

tritiated progestins and has been shown to have a high concentration of choline acetyltransferase (Fonnum et al., 1977; Palkovits & Jacobowitz, 1974; Whalen & Luttge, 1971). Within the MRF, it has been proposed that the majority of cholinergic receptors are muscarinic (Kuhar et al., 1975; Lake, 1973); nicotinic receptors have also been identified in the MRF (Morley et al., 1977).

The findings of the present study indicate that activation of the cholinergic neurons in the MRF, either with carbachol or with endogenous ACh (following administration of neostigmine bromide), has a facilitative effect upon sexual receptivity. These results raise the possibility that progesterone may achieve its facilitative effects upon lordosis by activation of cholinergic neurons. The present evidence suggests that in the MRF muscarinic and nicotinic receptors are involved in the facilitation of lordosis.

There are several lines of evidence which suggest that the MPOA may have its basis in normal estrogen-activation of sexual receptivity via cholinergic systems: (1) neurons in this region selectively concentrate estrogen (Pfaff & Keiner, 1973); (2) intracerebral implants of estrogen in this region facilitate lordosis (Lisk, 1962); (3) estrogen-pretreatment increases MPOA levels of ChAc, the enzyme responsible for synthesizing acetylcholine (Luine et al., 1975); and (4) muscarinic stimulation of the preoptic area induced high levels of sexual receptivity (Experiment 3). The facilitative effects following MPOA-muscarinic stimulation were of a longer latency than similar stimulation in the MRF (Experiment 3),

and unlike the MRF, nicotinic stimulation did not increase the probability of lordosis.

Failure of nicotinic stimulation within the MPOA to enhance sexual receptivity suggests that the facilitative effects of systemically administered nicotine (Fuxe et al., 1977) must reflect nicotinic action at sites other than, or in addition to, the anterior hypothalamus (e.g., the MRF). This supports the hypothesis of cholinergic regulation of sexual behavior in parts of the central nervous system other than the MPOA and the MRF.

These data taken together offer support for the idea that the estrogenic induction of lordosis is mediated in part by cholinergic neurons which are located in the MPOA. Within the MRF, however, cholinergic systems may participate in the synergism of estrogen and progesterone to facilitate sexual receptivity. These data suggest that the cholinergic system in the MRF may be composed of both muscarinic and nicotinic receptors.

In summary, the data in Part A are compatible with the notion that cholinergic mechanisms are involved in the mediation of sexual behavior at least at midbrain and hypothalamic synapses, and possibly at other sites as well.

## PART B. CENTRAL SEROTONERGIC SYSTEMS: POSSIBLE SEROTONERGIC INTERACTIONS WITH CENTRAL CHOLINERGIC SYSTEMS

The present series of experiments was designed to evaluate the effects of central serotonergic stimulation on female sexual behavior. In Experiment 1, females given low doses of estrogen were tested for facilitation of sexual behavior following intracerebral treatment with serotonergic antagonists. The females in Experiment 2 were treated with estrogen and progesterone and tested for inhibition of lordosis following intracerebral treatment with serotonin or serotonergic antagonists. Additionally, the effects of sequential cholinergic/serotonergic manipulations on female sexual behavior were ascertained (Experiment 3).

### General Methods

Details concerning animals, surgical and hormonal treatments, cannulation procedures, behavioral observation and testing procedures, and chemical stimulation techniques are described in Appendices A, B-1 and B-2, C, and D.

The general methods for Part B were identical to those described in Part A. Experimental females were selected on the basis of their ability to show sexual responses to suprathreshold doses of estrogen and progesterone. Receptivity tests consisted of

placing the female in a cage with a sexually active male rat until the male mounted the female 10 times. Lordosis frequency of the female in response to mounts by the male was used as the measure of sexual receptivity. This measure was expressed as a lordosis quotient (LQ: Lordosis Frequency/10 mounts x 100). Soliciting behaviors were also noted.

To administer serotonergic and cholinergic compounds into the brain, stainless steel cannulae were bilaterally implanted into ovariectomized Sherman strain female rats (see Appendices A and B).

One week after the implantation of cannulae, all females were injected with EB (1 μg/day) for three days. On the fourth day, females in Experiments 1 and 3 were given an injection of dexamethasone (125 μg/rat), and females in Experiment 2 received a progesterone injection (0.5 mg/animal). All females were tested for sexual receptivity four hr later; immediately following this test each female received an intrahypothalamic treatment. Inner cannulae were inspected at the conclusion of the last ten mount test, as in Part A.

## Experiment 1. Influence of Serotonergic Blockade in the MPOA-AH

### Methods

To determine whether serotonergic blockade in the estrogen-sensitive MPOA-AH would influence lordosis, 24 female rats were implanted bilaterally with cannulae in the MPOA-AH. The females were divided into two groups of 17 and 7 each (see below); each female received only one experimental treatment.

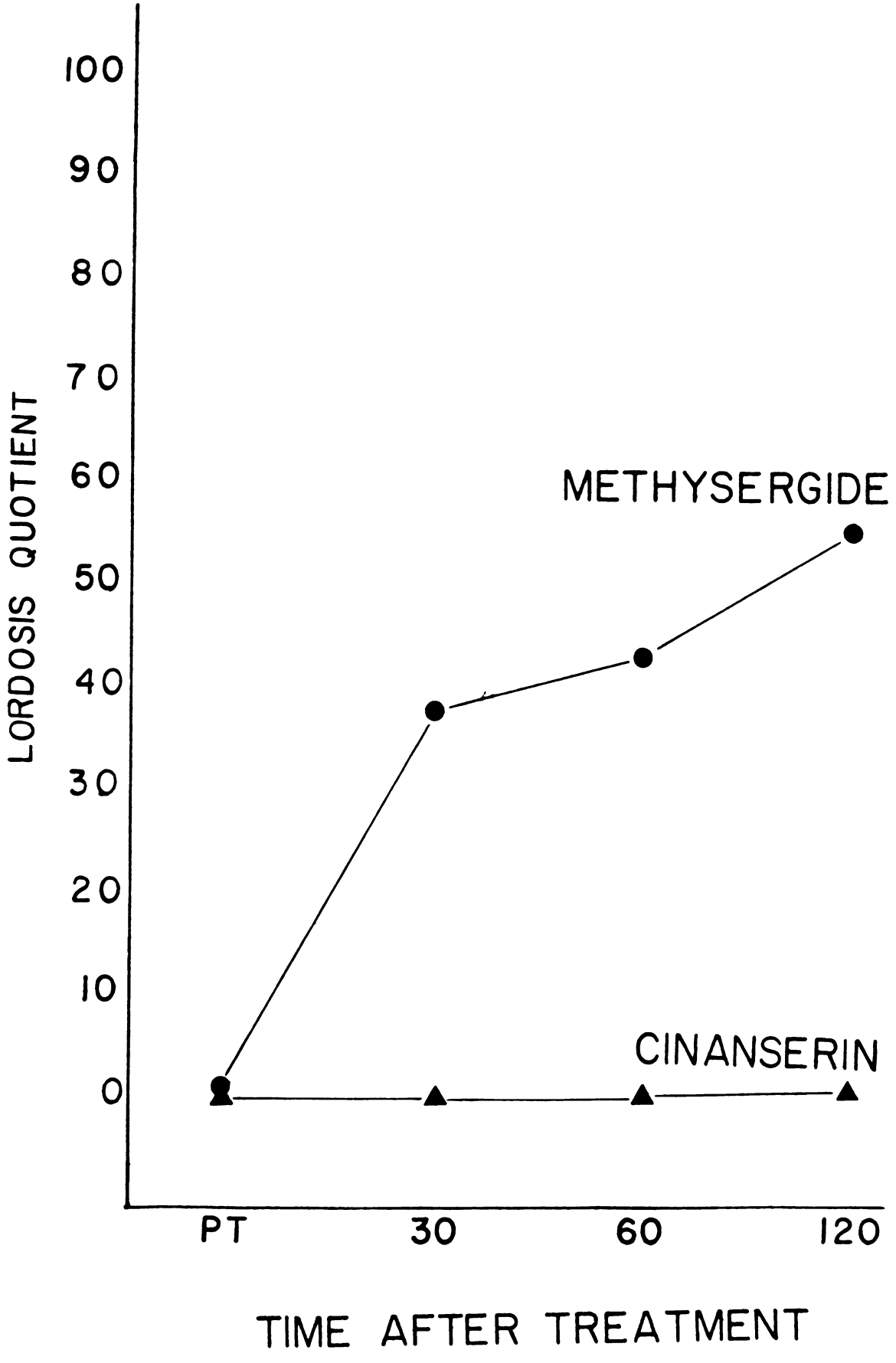
Beginning one week after implantation, each female was given the standard estrogen and dexamethasone pretreatment, followed by a 10 mount-pretest for lordosis. Immediately after the pretest, the females were treated with one of two serotonergic antagonists (Baldretti et al., 1965; Dombro & Wooley, 1964; Gyermak, 1961; Krieger & Rizzo, 1969; Proudfit & Anderson, 1973): methysergide maleate (10-15 µg/side; n = 17; Sandoz Chemical Co.) or cinanserin (10-15 µg/side; n = 7; Squibb). The animals were then tested 30, 60, and 120 min after intracerebral treatment.

Results

None of the animals achieved a lordosis during the pretest. Bilateral treatment of the MPOA-AH with methysergide resulted in a facilitation of lordosis in 12 of the 17 females. A significant increase in lordosis was observed during the 30, 60, and 120 min-tests after methysergide treatment ($p < .05$, Sign Test, for those females achieving at least one lordosis during the post-intracerebral treatment tests). Administration of cinanserin failed to facilitate lordosis ($p > .05$, Sign Test). Figure 6 shows the mean LQs on the pretest (PT) and on the repeated tests following intracerebral treatment with methysergide (lordosis-positive animals only; n = 12) or cinanserin.

Figure 7 shows the histological placement of the lordosis-positive and lordosis-negative methysergide implant sites. Eleven lordosis-positive sites were found in the MPOA and anterior hypothalamus. One additional lordosis-positive implant was found at

Figure 6.--Temporal changes in lordosis behavior following intracerebral administration of methysergide or cinanserin. Rats received EB (1 μg/animal/day, for three days) and dexamethasone four hours prior to ICT. Time in min. All values are means. Methysergide, ●——●; Cinanserin, ▲——▲.


100
90
80
70
60
50
40
30
20
10
0
LORDOSIS QUOTIENT
METHYSERGIDE
CINANSERIN
PT
30
60
120
TIME AFTER TREATMENT

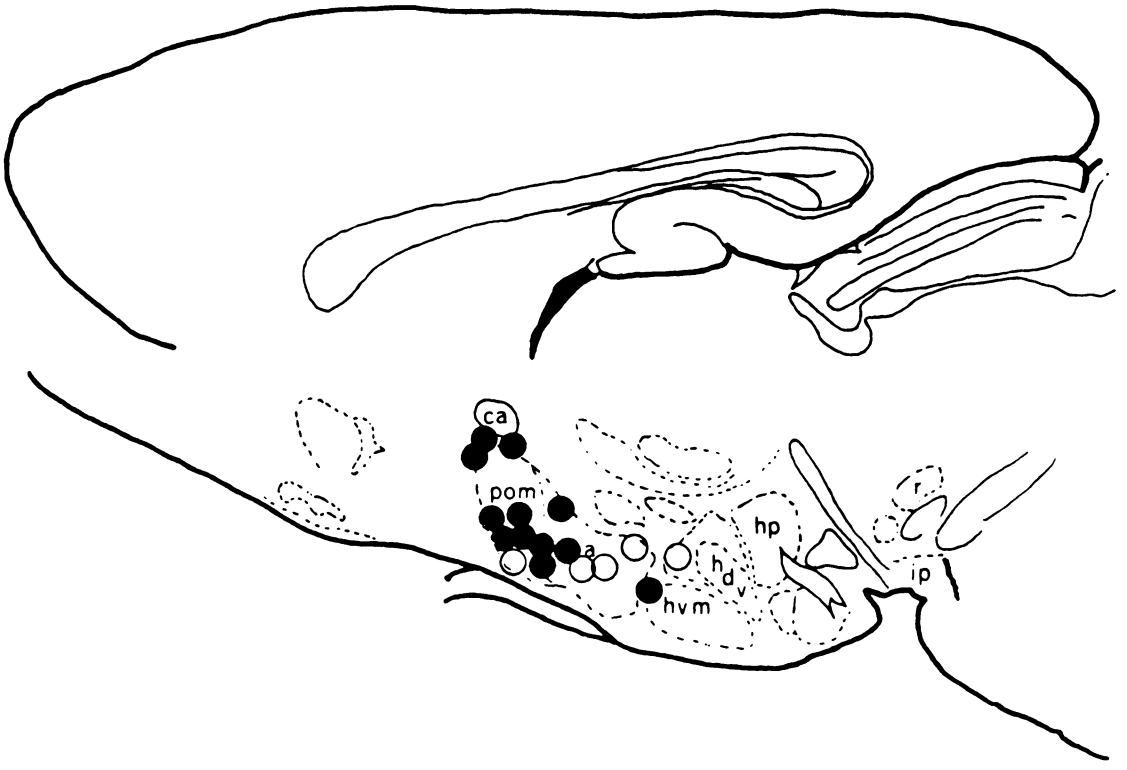



Figure 7.--Parasagittal view of the diencephalon and mesencephalon showing sites of methysergide implants in ovariectomized, EB-primed female rats. Lordosis-positive loci are indicated by the following symbol: (●). Lordosis-negative loci are indicated by the following symbol: (O). Lordosis-positive = LQ of 10 or greater on any post-intracerebral treatment test. Lordosis-negative = no lordotic behavior observed on any post-intracerebral treatment test. Abbreviations are the same as those in Figure 1.

the junction between the anterior hypothalamus and the ventromedial hypothalamus. The nonresponsive implant sites were found intermingled among the lordosis-positive sites: one negative site was found in the MPOA; three were in the anterior hypothalamus; and one was found immediately dorsal to the ventromedial hypothalamus.

Figure 8 shows the histological placement of the sites at which cinanserin administration failed to facilitate sexual receptivity. All implants were located within the MPOA.

## Experiment 2. Influence of Enhanced Serotonergic Neurotransmission or Serotonergic Blockade on Hormone-activated Sexual Behavior

### Methods

To determine whether enhanced serotonergic neurotransmission or serotonergic blockade inhibits estrogen/progesterone-activated sexual behavior, seven females were implanted bilaterally with cannulae in the MPOA. Coordinates were identical to those in Part A, Experiment 3.

Each female was tested once a week on three consecutive weeks following implantation. Prior to each test, the rats were pretreated with EB 1 μg/animal/day, for three days, followed by 0.5 mg of progesterone on the day of the test; this dosage of estrogen, followed by progesterone, normally induces high levels of sexual receptivity. The three intracerebral treatments were 5-HT (10-15 μg/side; Regis), cinanserin (10-15 μg/side), and methysergide (10-15 μg/side); the testing order of experimental conditions was randomized

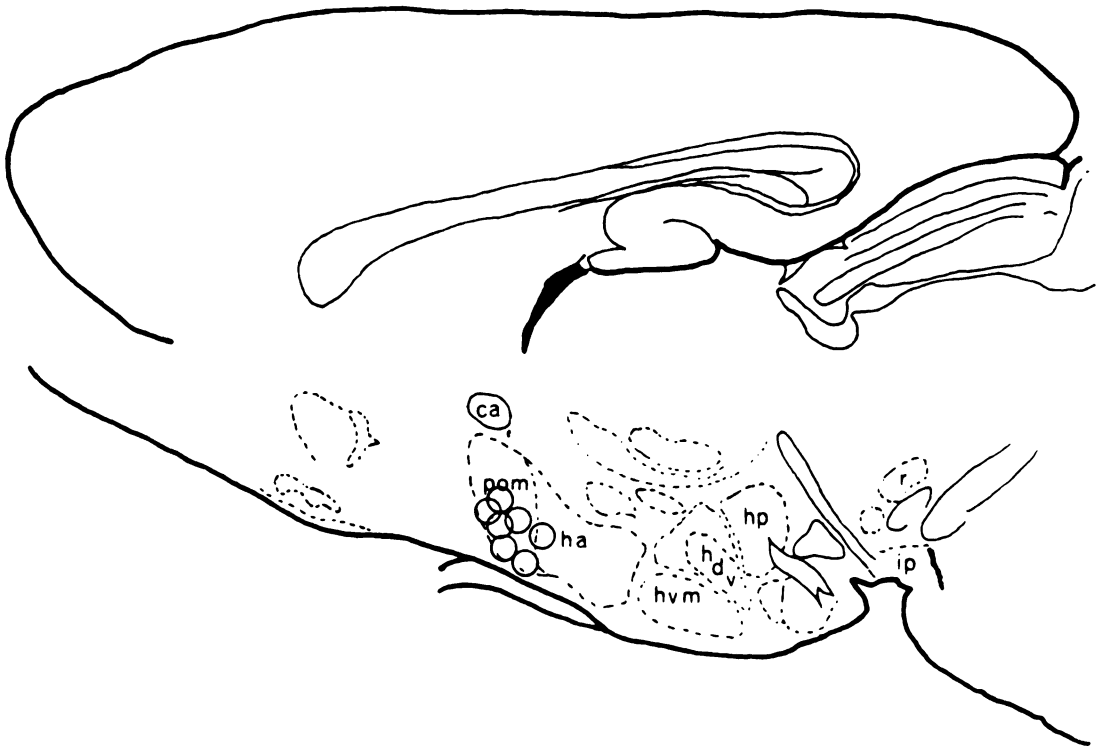



Figure 8.--Parasagittal view of the diencephalon and mesencephalon showing sites of cinanserin implants in ovariectomized, EB-primed female rats. Lordosis-negative loci are indicated by the following symbol: (O). Lordosis-negative = no lordotic behavior observed on any post-intracerebral treatment test. Abbreviations are the same as in Figure 1.

for each female. Following intracerebral treatment, the females were tested 30, 60, and 120 min later.

Results

Bilateral administration of 5-HT into the MPOA inhibited hormone-activated sexual behavior in all seven females ($F_{2,18}$ = 6.08, $p < .02$; Table 6). Individual comparisons between tests revealed that lordosis frequency was significantly decreased during the 30 and 60 min-tests following 5-HT administration ($p < .05$; Scheffés Test), but by the 120 min-test the levels of sexual receptivity had returned to a level which was not significantly different from the maximal pretest scores ($p > .05$; Scheffés Test; Table 6). Inhibition of serotonergic transmission following intracerebral administration of methysergide and cinanserin was without effect when compared to the pretest scores ($p > .05$; Scheffés Test; Table 6).

Figure 9 shows the histological placement of the 7 implant sites. All were found to lie within the MPOA.

## Experiment 3. Sequential Cholinergic/Serotonergic Manipulations and Female Sexual Behavior

Methods

In Part A it was reported that direct administration of cholinergic compounds carbachol, bethanechol, and neostigmine can facilitate lordotic behavior in estrogen-primed female rats; the effects of these compounds appear to be centrally mediated since they were inhibited by atropine sulfate (which readily penetrates

TABLE 6.--Mean LQs of EB/progesterone-primed female rats following intrahypothalamic treatment with methysergide, cinanserin, and serotonin. All values are means ± SEM.

| Treatment[a] | Post-treatment Time in Minutes | | | | | |
|---|---|---|---|---|---|---|
| | N | Pretest | 30 | 60 | 120 | P |
| Methysergide (10-15 µg) | 7 | 100.0 | 75.7 ± 13.9 | 75.7 ± 14.4 | 85.7 ± 14.3 | |
| Cinanserin (10-15 µg) | 7 | 100.0 | 71.4 ± 8.3 | 67.1 ± 16.4 | 77.0 ± 14.1 | |
| Serotonin (10-15 µg) | 7 | 100.0 | 7.1 ± 4.1* | 24.0 ± 10.6* | 64.0 ± 12.9 | * = P < .05 |

[a]Treatment; methysergide, cinanserin, and serotonin: (intracerebral).

*Pretest scores versus scores during the 30, 60 and 120 min. tests.

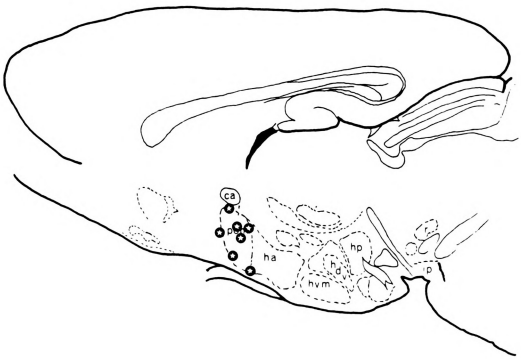



Figure 9.--Parasagittal view of the diencephalon and mesencephalon showing sites of implants of serotonin, methysergide, and cinanserin in ovariectomized, EB-primed female rats. Cannulae tips are indicated by the following symbol (*). Abbreviations are the same as in Figure 1.

the brain). The results of experiments in which cholinergic compounds were administered systemically suggest that muscarinic stimulation inhibits lordosis via a serotonergic mechanism (Lindstrom, 1970; 1971). The results of Experiment 1 and 2 (Part B) offer support for the suggestion that serotonin exerts a marked inhibitory influence on lordotic behavior. Thus, the purpose of Experiment 3 was to give additional information of the types of central receptors which may be involved in mediating the actions of serotonergic antagonists. In order to concurrently inhibit serotonergic and cholinergic receptors, methysergide and cinanserin (serotonergic antagonists) were administered in combination with hexamethonium (nicotinic antagonist) and atropine (muscarinic antagonist). By administering these drugs in this manner possible interactions between serotonergic and cholinergic neuronal systems could be ascertained.

Using this treatment protocol it was felt it would be possible to assess whether the actions of serotonergic antagonists on female sexual behavior could be enhanced or reduced by altering cholinergic receptor activity.

Fourteen female rats were bilaterally implanted in the MPOA with cannulae. Coordinates for the MPOA implants were identical to those in Part A. These female rats were divided into two groups of 7 each; a methysergide group (M) and a cinanserin group (C). Each female was tested once a week for three consecutive weeks; prior to each experimental test females were pretreated with EB and dexamethasone as in Part A.

One hour prior to pretesting each female was infused with either 0.5 µl of atropine sulfate (26 µg/µl, Fisher) to block muscarinic receptors; 0.5 µl of hexamethonium chloride dihydrate (56 µg/1.0 µl/side; hexamethonium; Mann) to block nicotinic receptors; or with 0.5 µl of the vehicle (artificial cerebrospinal fluid pH 7.4). All treatments with atropine, hexamethonium, or vehicle were randomized within groups, i.e., C or M. Any female achieving an LQ of 30 or above in the pretest was considered to be receptive, and was not used in the experiment.

Immediately following the pretest, crystalline methysergide (10-15 µg/side) or cinanserin (10-15 µg/side) was loaded into the cannula as described in Part A. All females were then tested 30, 60, and 120 min later. At the completion of testing inner cannula were inspected as in previous experiments. At the conclusion of the experiment all females were sacrificed and their brains processed for histological examination.

Details concerning infusion procedures and vehicle preparation may be found in Appendix D. Briefly, pharmacological antagonists (atropine and hexamethonium) were infused via a 27 guage injection cannula which was lowered through the outer guide cannula. A 0.5 µl volume of the infusion fluid was delivered through calibrated PE tubing by a Harvard Apparatus infusion/withdrawal pump to the unanesthetized female rat. Infusions lasted 30 sec, at which time the injection cannula was removed 15 sec later, and a blank indwelling cannula replaced. This procedure was accomplished with

little apparent stress to the rats. Infusates were prepared daily in pyrogen-free, five ion-artificial cerebrospinal fluid (Appendix D).

Results

Analysis of variance revealed that sequential bilateral anticholinergic and anti-serotonergic stimulation of the MPOA facilitated lordosis behavior ($F_{2,24} = 7.98$, $p < .001$). The time of testing was also observed to be significant ($F_{3,36} = 34.68$, $p < .001$); that is, lordosis scores increased with time after intrahypothalamic treatment.

Individual comparisons for cinanserin (Scheffés Test) revealed that the drug treatment effect was due primarily to the effect of hexamethonium pretreatment rather than atropine (Table 7). The drug effect increased with time; a significant increase in lordosis was observed 60 and 120 min after sequential intracerebral treatment with cinanserin (Table 7; $p < .01$; Pretest (PT) scores versus scores from 60 and 120 min-tests). Cinanserin was also observed to be more effective following pretreatment with hexamethonium than with the vehicle ($p < .01$; Scores from cinanserin and vehicle versus cinanserin and hexamethonium, for the 60 and 120 min tests).

Methysergide had an effect similar to, but less reliable than, sinanserin when combined with the cholinergic drug treatment. Animals treated with hexamethonium and methysergide scored significantly higher during the 120 min-test than during the pretest ($p < .01$; Table 8). When compared with hexamethonium and vehicle

TABLE 7.--Mean LQs of EB-primed female rats following intrahypothalamic (MPOA) treatment with cinanserin in combination with vehicle, atropine, or hexamethonium. All values are means ± SEM.

| Treatment[a] | | Post-treatment Time in Minutes | | | | |
|---|---|---|---|---|---|---|
| | N[b] | Pretest | 30 | 60 | 120 | P |
| Cinanserin (10-15 μg) and Vehicle (saline, .5 μl) | 7 | 0.0 | 0.0 | 0.0 | 0.0 | |
| Cinanserin (10-15 μg) and Atropine | 7 | 0.0 | 28.5 ± 18.4 | 28.5 ± 18.4 | 41.4 ± 16.7 | |
| Cinanserin (10-15 μg) and Hexamethonium | 7 | 1.0 | 24.2 ± 13.4 | 41.1 ± 19.0*[c] | 85.7 ± 10.3*[c] | * = P < .01 |

[a]Treatments: Cinanserin (intrahypothalamic; crystalline); Atropine Sulfate (28 μg/μl; intrahypothalamic; solution); Hexamethonium Chloride Dihydrate (56 μg/μl; intrahypothalamic; solution).

[b]Includes animals which achieved a lordosis quotient of 10 or greater in one of the tests following intrahypothalamic treatment.

[c]*Scheffes Test; Pretest scores versus scores from 60 and 120 min. tests.
Scheffes Test; Scores from cinanserin and vehicle tests versus scores from cinanserin and hexamethonium tests during 60 and 120 min. tests.

TABLE 8.--Mean LQs of EB-primed female rats following intrahypothalamic (MPOA) treatment with methysergide in combination with vehicle, atropine, or hexamethonium. All values are means ± SEM.

| Treatment[a] | | Post-treatment Test Time in Minutes | | | | |
|---|---|---|---|---|---|---|
| | N[b] | Pretest | 30 | 60 | 120 | P |
| Methysergide (10-15 µg) & Vehicle (saline, .5 µl) | 7 | 1.4 | 18.5 ± 13.8 | 15.7 ± 11.0 | 31.4 ± 17.8 | |
| Methysergide (10-15 µg) & Atropine (28 µg/µl) | 7 | 0.0 | 14.2 ± 12.6 | 14.2 ± 12.7 | 34.3 ± 17.8 | |
| Methysergide (10-15 µg) & Hexamethonium (56 µg/µl) | 7 | 0.0 | 0.0 | 20.0 ± 7.8 | 100.0[c*] | *=P < .01 |

[a]Treatment; methysergide (intrahypothalamic, crystalline); atropine sulfate (intrahypothalamic, solution); hexamethonium chloride dihydrate (intrahypothalamic, solution).

[b]Includes animals which achieved a lordosis quotient of 10 or greater in one of the tests following intrahypothalamic treatment.

[c]*Scheffes Test; Pretest scores versus scores from 120 min. tests.
Scheffes Test; Scores from methysergide and vehicle 120 min. test versus scores from the methysergide and hexamethonium 120 min. test.

test scores, female rats treated with hexamethonium and methysergide scored significantly higher (Table 8; $p < .01$).

Intrahypothalamic treatment with cinanserin (Table 7) or methysergide alone (Table 8), or sequentially with atropine sulfate failed to influence the frequency of lordosis ($p > .05$). The results of preliminary experiments indicated that hexamethonium, atropine, or the vehicle control alone were without significant effect upon lordosis (Humphrys, personal observations).

Figure 10 shows the histological placement of these implant sites; methysergide implant sites are indicated by an asterisk (*), and cinanserin implant sites are indicated by filled circles. All implant sites were located in the MPOA.

## Discussion

The original suggestion that 5-HT subserves an inhibitory role (Meyerson, 1964a) on female sexual behavior received support from the experiments in Part B. Inhibition of serotonergic neurotransmission (methysergide, but not cinanserin) moderately facilitated sexual behavior in estrogen-primed female rats (Experiment 1), while enhancement of serotonergic neurotransmission (with 5-HT) transiently inhibited estrogen/progesterone-activated sexual behavior (Experiment 2). These data are consistent with a variety of pharmacological evidence which suggests that 5-HT mediates behavioral inhibition, possibly at the level of the MPOA. In addition, sequential inhibition of both serotonergic and nicotinic processes increased lordosis frequency (Experiment 3).

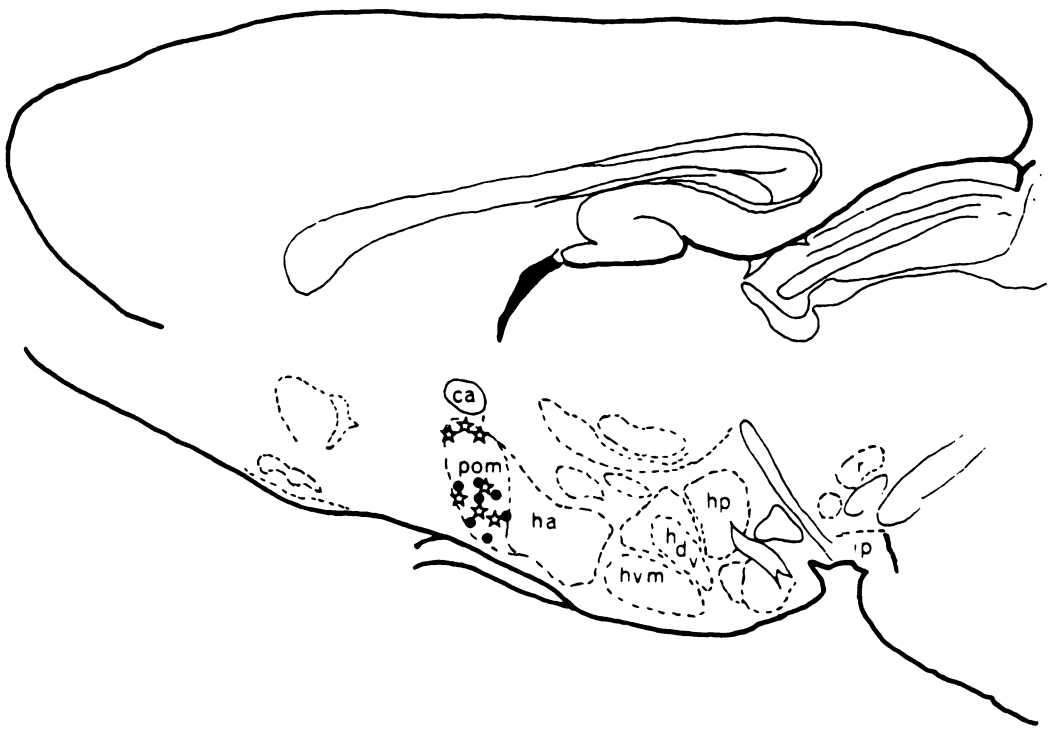



Figure 10.--Parasagittal view of the diencephalon and mesencephalon showing sites of methysergide and cinanserin implants in EB-primed female rats. Implant loci indicated by the following symbol are for methysergide implants (*). Implant loci indicated by the following symbol are for cinanserin implants (●). Abbreviations are the same as those for Figure 1.

One aspect of the suggestion that 5-HT suppresses sexual behavior is that decreases in central serotonergic neurotransmission should facilitate sexual receptivity. The finding that the central application of methysergide (cinanserin was without effect) failed to reliably induce high levels of receptivity in all females is, however, inconsistent with this suggestion. The failure of cinanserin to facilitate sexual receptivity may be related to differences between methyserigide and cinanserin and the time of onset of these drugs central effects. For example, cinanserin, unlike methysergide, has been observed to require at least 2 hr to antagonize central serotonergic receptors (Rech, personal communication). There are several other likely explanations for this discrepancy. Second, the blocking action of methysergide and cinanserin may be too temporary to have effects upon sexual behavior at the post-injection intervals used in the present experiments. While almost all of the direct evidence for the post-synaptic blocking action of these compounds comes from work in the peripheral nervous system (Baldretti et al., 1965; Dombro & Wooley, 1964; Gyermak, 1961; Krieger & Rizzo, 1969; Proudfit & Anderson, 1973), it has been demonstrated recently that these serotonergic antagonists have weak central antagonistic actions (Everitt et al., 1975b), but are not always effective centrally (Haigler & Aghajanian, 1973). Third, diffusion of methysergide and cinanserin within the anterior hypothalamus may have been insufficient to block enough relevant neural elements; this, however, is unlikely considering the high dosage of drug administered. Fourth, there may be such a redundancy of circuitry

in the brain related to sexual receptivity that a functional blockade of anterior hypothalamic serotonergic systems alone is not sufficient to reliably elicit high levels of sexual receptivity. The results of Experiment 3, which demonstrated that blockade of serotonergic receptors synergized with blockade of nicotinic receptors to facilitate sexual behavior, would support the latter conjecture. That is, the synergistic effects suggest that more extensive blockade of additional (inhibitory) transmitter systems, rather than antagonism of solely serotonergic systems, is necessary to reliably facilitate sexual receptivity.

An alternative interpretation of the increased lordosis frequency seen following combined serotonergic and nicotinic blockade is that this combined treatment activated the adrenal gland. Activation of the adrenal gland may have resulted in an increased secretion of endogenous progestins which then facilitated lordosis. To minimize the possibility of adrenal involvement, all the animals were pretreated with dexamethasone to reduce adrenal progestin secretion (Paris et al., 1971). However, with no independent measures of systemic progestin levels, the possible influence of adrenal progestin cannot be excluded.

The only report in the literature of facilitative effects of serotonergic antagonists administered intracerebrally in estrogen-primed female rats (Ward et al., 1975) was not completely replicated in the present experiment. The reasons for the failure to replicate Ward et al. (1975) are not clear. Possibly differences in experimental procedures were involved; for example, bilateral (the present

study) versus unilateral cannulation; testing criteria may have been different; and the dosages of the drugs were different. The lower lordosis scores obtained in the present study may indicate that the behavioral responses to the serotonergic antagonists are related to estrogen dosage. This is suggested by the fact that in the present study, all animals were pretreated with 3 µg of EB, whereas in Ward's study, all animals received 10 µg of EB.

At the present time it would appear that in the estrogen-primed female rat, elements within the MPOA have the potential to exert a facilitative influence upon the probability of lordosis via neural systems which are subserved by muscarinic receptors (Part A). In addition, elements within the MPOA of the estrogen-primed female also appear to have nicotinic and serotonergic systems which may be capable of maintaining a tonic inhibitory influence over the lordotic reflex.

## PART C. THE INFLUENCE OF CATECHOLAMINES IN MEDIATING FEMALE SEXUAL BEHAVIOR

The present studies were designed to permit an evaluation of the effects of central alpha- and beta-adrenergic stimulation on female sexual behavior. Additionally, the influence of dopaminergic and adrenergic systems in mediating estrogen/progesterone-activated sexual behavior was examined.

### General Methods

Details concerning animals, surgical and hormonal treatment, cannulation procedures, behavioral observation and testing procedures, and chemical stimulation techniques are described in Appendices A, B-1 and B-2, C, and D.

The general methods for Part C were identical to those of Part A. Selection procedures for females, receptivity test criteria, and histological procedure were as those described in Experiment 1, Part A, pages 24-27.

### Experiment 1. Contributions of Alpha- and Beta-adrenergic Systems

#### Methods

To determine whether beta-adrenergic blockade alone, or sequentially following stimulation of alpha-adrenergic mechanisms in the MPOA would facilitate lordosis, eight female rats were

bilaterally implanted with cannulae. Coordinates were the same as those given in Experiment 3, Part A.

Each female was tested once a week for four consecutive weeks; in each test the female rats were treated intracerebrally with 9-12 µg/side of crystalline dl-4-(2-hydroxy-3-iso-propylamino-propoxy)-indole (LB-46; Sandoz Laboratories), to block beta-adrenergic receptors (Giudicelli et al., 1969); l-epinephrine (12 µg/0.5 µl/side; Epinephrine; Calbiochem), to stimulate alpha-adrenergic receptors (Goodman & Gilman, 1970); 0.5 µl of artificial cerebrospinal fluid/side; or both LB-46 and epinephrine. All treatments were randomized for each female. Infusion procedures for epinephrine and vehicle administration were as those described in Part B, Experiment 3, and in Appendix D.

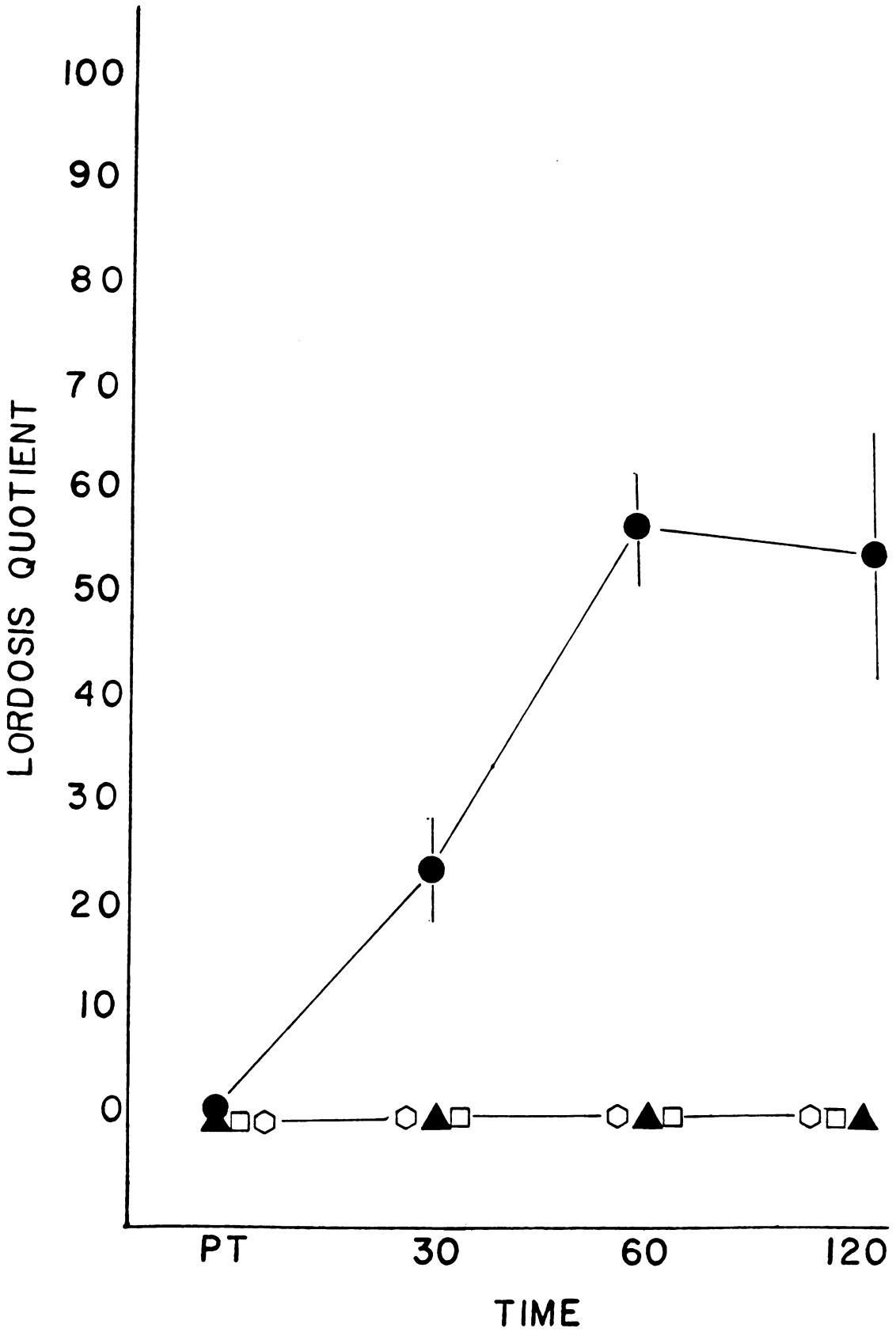
All females were given the standard estrogen and dexamethasone pretreatment. On those tests in which epinephrine or the vehicle was administered, they were infused 60 min prior to pretesting; LB-46 was administered, on appropriate tests, immediately after the pretest. All animals were tested for receptivity 30, 60, and 120 min after the pretest.

Results

Sequential, bilateral administration of epinephrine and LB-46 into the MPOA facilitated lordotic behavior in 6 of the 9 females ($F_{3,8} = 79.16$, $p < .001$).

Figure 11 shows the mean LQs on the pretest and on repeated tests following sequential intracerebral treatment with LB-46 and

Figure 11.--Temporal changes in lordosis behavior of EB-primed female rats following sequential intracerebral administration of LB-46 and Epinephrine, LB-46 alone, Epinephrine alone, and vehicle. Time in min. All values are means. LB-46 and Epinephrine, ●———●; LB-46, ⬡———⬡; Epinephrine, △———△; Vehicle, □———□.


100
90
80
70
60
50
40
30
20
10
0
LORDOSIS QUOTIENT
PT
30
60
120
TIME

epinephrine, LB-46 alone, epinephrine alone, and the vehicle alone. Meximal levels of responding were observed 60 min after combined treatment with LB-46 and epinephrine. Individual comparisons of the means revealed that the combined treatment significantly increased the lordosis frequency during the 60 and 120 min-tests ($p < .05$; Scheffés Test, PT scores versus scores from 60 and 120 min-tests). Intracerebral treatment with LB-46 alone, epinephrine alone, or the vehicle was without effect upon lordosis ($p > .05$; Scheffés Test, PT scores versus scores from the 30, 60, and 120 min-tests). None of the experimental treatments produced any obvious adverse drug effects.

Figure 12 shows the histological placement of the lordosis-positive and lordosis-negative implant sites. The lordosis-positive sites ($n = 6$) were found within the MPOA; one nonresponsive implant site was found in the MPOA, while the other site was located within the anterior hypothalamus.

## Experiment 2. Influence of Dopaminergic and Adrenergic Systems in Mediating Hormone-activated Sexual Behavior

### Methods

To determine whether enhanced adrenergic or dopaminergic neurotransmission inhibits estrogen-progesterone-activated sexual behavior, five female rats were implanted bilaterally with cannulae in the MPOA. Coordinates and experimental procedures were the same as those described for Experiment 2, Part B.

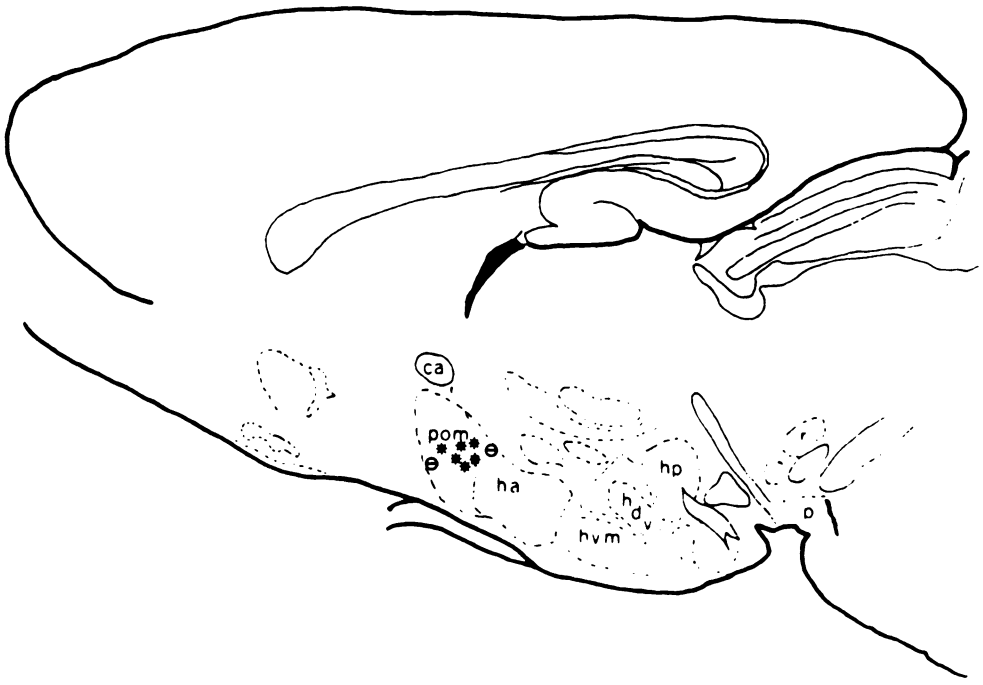



Figure 12.--Parasagittal view of the diencephalon and mesencephalon showing sites of alpha- and beta-adrenergic implants in EB-primed female rats. Lordosis-positive loci are indicated by the following symbol (*). Lordosis-negative loci are indicated by the following symbol (⊖). Lordosis-positive = LQ of 30 or greater on any post-intracerebral test following administration of LB-46 and epinephrine. Lordosis-negative = LQ of 0-20 on any of the post-intracerebral treatment tests. Abbreviations are the same as in Figure 1.

Each female was tested once a week for 2 weeks, with either dopamine (10-15 µg/side; DA; Smith, Kline and French), or with norepinephrine (10-15 µg/side; NE; Calbiochem). Treatment order was randomized for each female. On each test all females were given a standard estrogen-pretreatment, followed by 0.5 mg progesterone. Four hours later, immediately prior to intracerebral treatment, each female was given a pretest for sexual receptivity. Following intracerebral treatment, each female was tested 30, 60 and 120 min later.

Results

Intracerebral treatment with NE significantly reduced lordosis frequency ($F_{1,8} = 82.36$, $p < .001$; Figure 13). Analysis of the mean LQs revealed that the lordosis frequency was significantly decreased on all tests following NE administration ($p < .05$; Scheffés Test; Figure 13). Although the mean levels of responding were reduced following treatment with DA, the differences did not reach statistical significance ($p > .05$; Scheffés Test; PT scores versus scores from 30, 60, and 120 min-tests). Figure 14 shows the histological placement of the implant sites. All five were found to lie within the MPOA.

## Discussion

The results of Part C demonstrated that inhibition of beta-adrenergic receptors (with LB-46) following stimulation of alpha-adrenergic receptors (with epinephrine) within the MPOA can stimulate sexual receptivity in estrogen-primed female rats. Inhibition of beta-adrenergic receptors or stimulation of alpha-adrenergic

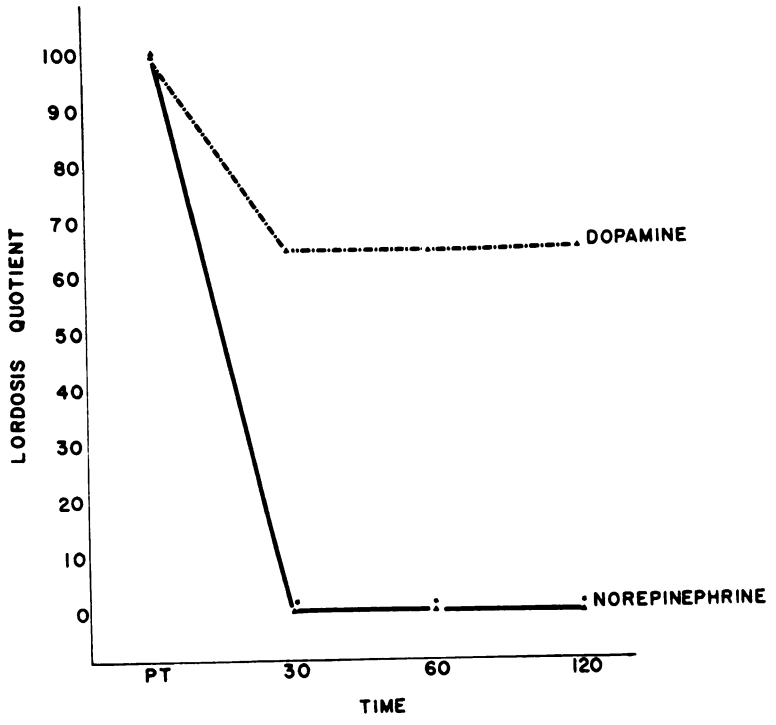



Figure 13.--Temporal changes in lordosis behavior following ICT with norepinephrine and dopamine. All values are means. Time in min.

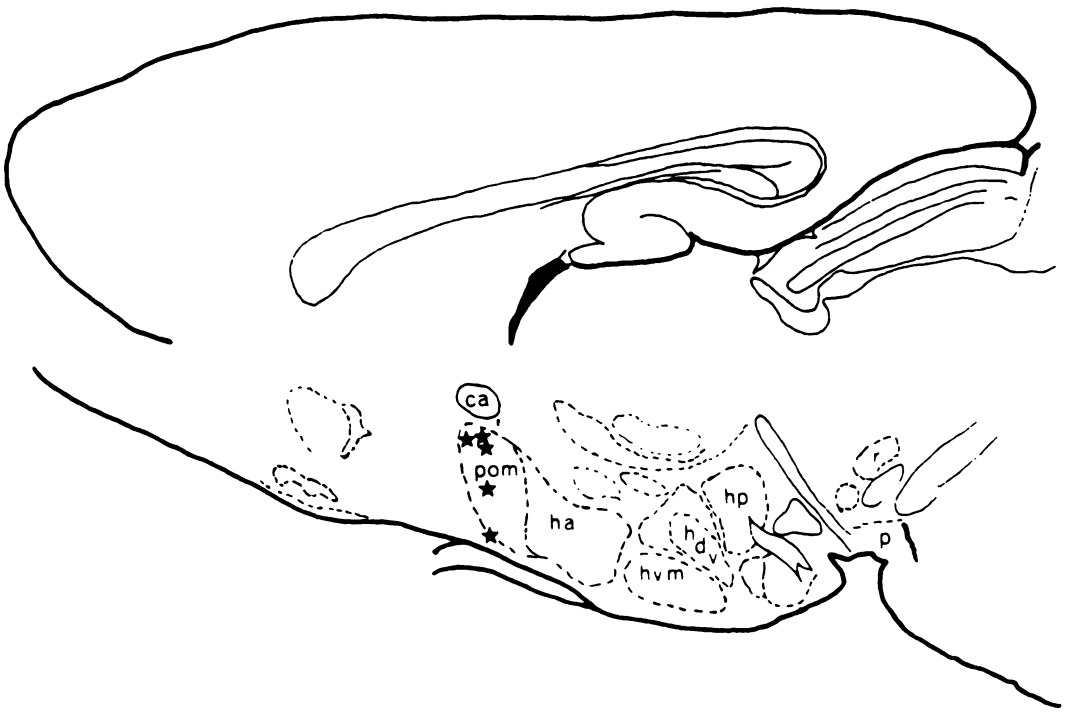



Figure 14.--Parasagittal view of the diencephalon and mesencephalon showing sites of dopamine and norepinephrine implants in EB/progesterone primed female rats. Implant sites are indicated by (*). Abbreviations are the same as those in Figure 1.

receptors alone was ineffective in facilitating lordosis. It was observed that NE, but not DA, suppressed lordotic responding in female rats brought into estrus by estrogen and progesterone.

It has been suggested that inhibition of beta-adrenergic receptors facilitates sexual receptivity in EB-primed female rats (Ward et al., 1975). This suggestion was based upon the results of experiments in which the probability of lordotic responding increased following intrahypothalamic treatment with LB-46 in the MPOA. The present results are not entirely consistent with this suggestion, since LB-46 alone failed to facilitate lordosis. Differences in implant procedures (bilateral vs. unilateral), testing criteria, and dosages of drugs and estrogen (3 μg vs. 10 μg) may contribute to these discrepant results.

The present data (Experiment 1) support the hypothesis that the facilitative effects of beta/alpha-adrenergic manipulations may be mediated by the pituitary gland. For example, only sequential drug administration (beta-adrenergic blockade combined with alpha-adrenergic stimulation) facilitated sexual receptivity. Despite the presence of dexamethasone, stimulation of the pituitary-adrenal axis may be a viable mechanism by which these combined intracerebral treatments facilitated receptivity. Alternatively, the combined effect of these treatments may have been exerted via a luteinizing hormone-releasing hormone (LH-RH)-mediated mechanism. This suggestion is supported by the observation that LH-RH can facilitate sexual receptivity in estrogen-primed female rats (Pfaff, 1973),

and that alpha-adrenergic stimulation potentiates the release of LH-RH (Schneider & McCann, 1970).

The results of investigations using systemic drug administration have suggested specific and separable roles for the catecholamines (i.e., epinephrine, norepinephrine, and dopamine) in the control of sexual receptivity by estrogen and progesterone. For example, it was observed that drugs which reduce dopamine (Everitt et al., 1975a), epinephrine (Everitt et al., 1975a), or alpha-adrenergic receptor activity (Davis and Kohl, 1977), or increase adrenergic neurotransmission (Everitt et al., 1975a) tended to facilitate sexual receptivity. Drugs having the opposite effects inhibited sexual receptivity induced by estrogen and progesterone (Everitt et al., 1975a, b; Everitt & Fuxe, 1977).

The results of the present experiments, in which drugs were administered intracerebrally, are not entirely consistent with those cited above, in which the drugs were administered systemically. Discrepancies such as these may reflect the dissimilarity between the central and peripheral effects of catecholamine administration, and indicate the need for anatomical localization of drug effects. Other examples which demonstrate the need for studies of local drug effects may be found in the lterature. For example, epinephrine is a powerful anorexigenic compound when given systemically (Russek et al., 1967), but when injected into circumscribed sites in the brainstem, this same catecholamine evokes intense feeding behavior in the rat (Booth, 1969; Grossman, 1960; Leibowitz, 1970). Likewise, when administered systemically, epinephrine is a very potent

thermogenic compound (Myers, 1974); when administered into the anterior hypothalamus of the cat (Rudy & Wolf, 1971) or the monkey (Myers & Yaksh, 1969), this catecholamine produces a dramatic fall in basal body temperature. These examples and the results of Part C, which contrast with the results of previous studies using systemic drug administration, illustrate the importance of anatomical localization of drug effects.

The mechanism by which intracerebral NE resulted in the suppression of sexual receptivity, observed in the experiments in Part C, may be the result of administering the compound intracerebrally. Central administration of NE can reduce flood flow within specific brain areas (Rosendorf & Cranston, 1971). Thus, the resultant suppression of sexual behavior may have involved decreased blood flow as a result of vasoconstriction and an increase in vascular resistance (Goodman and Gilman, 1970). Consequently, it may be hypothesized that catecholamine-induced vascular changes may have precipitated ischemia in various brain regions. The neuronal reactions to this ischemia may have resulted in the observed reduction of sexual receptivity. Future experiments which are designed to ascertain the role of catecholamines in the regulation of sexual receptivity should include an analysis of open-field activity; this would aid in determining whether behavioral deficits are due to lethargy, dysfunction of motor systems, or a general decrease in activity. Without these behavioral measures, it is difficult to determine whether the deficits in sexual behavior observed after intracerebral treatment with ME were due to ischemia and subsequent

neuronal damage, or to direct modulation of brain regions subserving sexual behavior.

An alternative explanation for the inhibition of estrogen/progesterone-activated sexual behavior observed following NE treatment may involve selective stimulation of inhibitory post-synaptic receptors. A similar mechanism has been proposed to account for the inhibition of estrogen/progesterone-activated sexual behavior following dopaminergic stimulation (Everitt & Fuxe, 1977). Everitt and Fuxe (1977) reported that receptivity was inhibited following stimulation of post-synaptic dopaminergic receptors; stimulation of pre-synaptic dopamine receptors was without effect. It was suggested that catecholamines may inhibit hormone-induced sexual behavior via inhibitory post-synaptic (i.e., dopaminergic) receptors (Everitt & Fuxe, 1977). The results of Experiment 3 do not offer support for such a suggestion. As noted in the Results (Experiment 3), intracerebral treatment with DA in the MPOA did not affect estrogen/progesterone-activated sexual receptivity. However, preliminary evidence suggests that small quantities of DA and NE (presumably stimulating facilitative pre-synaptic receptors) infused directly into the MPOA facilitates sexual receptivity in estrogen-primed female rats (Clemens, personal communication, 1977).

# GENERAL DISCUSSION

It is well established that sexual behavior in the female rat is under the control of the ovarian hormones estrogen and progesterone (Beach, 1942; Davidson, 1972; Young, 1961). However, the physiological bases for the actions of estrogen, and the synergism of estrogen with progesterone, are unknown at the present time. A large body of evidence has accumulated which suggests that biogenic amines may mediate the effects of hormones on female sexual behavior (Everitt, 1975; Meyerson & Malmnas, 1977). These data were derived from experiments in which it was observed that systemically administered drugs which affect aminergic transmission influence the actions of hormones (e.g., progesterone).

The results of the present series of experiments have provided more evidence which indicates that neurotransmitters may participate in the regulation of female sexual behavior. The results of Part A indicated that cholinergic implants within the basal MPOA or the MRF region can stimulate high levels of sexual receptivity in estrogen-primed, ovariectomized, female rats. It was concluded that: (1) acetylcholine was involved in the regulation of female sexual behavior by influences within the MPOA and the MRF; (2) stimulation of both muscarinic and nicotinic receptors within the MRF facilitated sexual receptivity, whereas within the MPOA only

muscarinic receptor stimulation was effective; and (3) stimulation of the pituitary-adrenal axis, and the release of adrenal progesterone, was not responsible for the increase in lordosis frequency following cholinergic stimulation.

The findings that lordosis behavior was facilitated in estrogen-primed female rats following intracerebral treatment with the serotonergic antagonist methysergide, and that central administration of 5-HT blocked estrogen/progesterone-activated sexual behavior, offer support for the concept that serotonin suppresses female sexual behavior (Part B). The observation that concurrent antagonism of serotonergic and nicotinic mechanisms within the MPOA facilitated sexual receptivity, while either alone was ineffective, led to the tentative conclusion that redundant pathways (e.g., nicotinic and serotonergic) may exist which interact to tonically inhibit female sexual behavior.

The results of Part C demonstrated that sequential implants of alpha-adrenergic agonists and beta-adrenergic antagonists can facilitate sexual receptivity in estrogen-primed female rats. It was concluded that, within the MPOA, redundant pathways are available which facilitate lordosis (e.g., alpha-adrenergic and muscarinic systems). The findings that NE but not DA could abolish hormone-activated sexual behavior led to the tentative proposal that NE, in addition to 5-HT, may inhibit female sexual behavior.

The results of the present series of experiments raise several questions for future investigations, and have implications

for our understanding of the neuropharmacological control of female sexual behavior.

The first question which should be considered concerns the localization of the neurotransmitter mechanisms which appear to participate in the modulation of sexual behavior. Although the implants in Part A, B, and C were placed in the medial preoptic-anterior hypothalamus and/or the midbrain reticular formation, diffusion throughout the adjacent ventricular or vascular systems could have resulted in the drugs exerting an effect in a variety of subcortical regions. Non-specific diffusion has been suggested as an explanation of the effects of intracerebrally administered cholinergic and anti-cholinergic drugs on water consumption in the rat, and on affective aggression in the cat (Myers, 1974).

That non-specific diffusion may not account for these results (Part A) is suggested by the results of experiments in which the physical spread of chemical substances from the site of injection was investigated. For example, when a small injection volume (1 µl or less) is used (crystalline material shows a pattern of spread in neural tissue that is nearly identical with that of a solution) a spread of approximately 1 mm in diameter can be expected to occur at the injection site (Myers, 1974; Leibowitz, 1978). This finding is supportive of the behavioral results (observed in Part A) demonstrating clear differences in responsiveness with 1-2 mm shifts in the injection site. However, the pattern of spread may be expected to vary depending on the drug injected, as well as the sites of injection.

Concerning the localization of the chemicals' effects, and the identification of the effective neural structures, the influence of nicotinic systems within the MPOA upon female sexual behavior deserves further consideration. In contrast to the effects of intracerebral treatment with bethanechol (Part A, Experiment 3), the data concerning the effects of nicotinic stimulation offer support for the suggestion that nicotinic stimulation within the MPOA exerts an inhibitory influence on sexual receptivity, while stimulation of nicotinic receptors in the MRF appears to increase the probability of lordosis (Experiment 3, Part A).

Future experiments should include: (1) intraventricular implants, in order to ascertain the effects of extensive drug diffusion; and (2) implants of neurotransmitter substances dorsal to positive loci, to control for the spread of the drug upward along the cannula shaft. These experiments would aid in the localization of the chemicals' effects, and the identification of the effective neural structure(s), involved in the elaboration of sexual receptivity in the female rat.

Thus, until extensive mapping and control experiments are completed there may be a need to postulate extensive drug diffusion in order to explain the facilitation of sexual behavior following intracerebral treatment.

A second point concerns the use of control conditions to assess the possibility that intracerebrally administered drugs may have exerted their effects on sexual behavior by: (1) producing local osmotic changes; (2) changing local concentrations of

inorganic ions; and (3) producing changes in local electrical activity which may result in the production of focal seizures in subcortical regions. That care should be taken to avoid confounding behavioral results with the effects of production of abnormal osmotic conditions or changes in inorganic ions is suggested by the observation that local changes in cation (i.e., calcium) concentrations result in the facilitation of lordosis (Humphrys, personal observations). The effects of intracerebrally administered drugs (especially those which affect cholinergic mechanisms) on the electrical activity of cells adjacent to implant sites would be more difficult to evaluate than changes in osmotic conditions or inorganic ions. However, in view of acetylcholine's well-known participation in the mediation of seizure activity, caution should be taken to avoid confounding behavioral results with seizure artifacts (Myers, 1974). The results of a preliminary study (Kent, 1972), however, suggest that intracerebral administration of neurotransmitters (e.g., ACh, NE, and Epinephrine) does not alter the normal electrical activity of cells in the vicinity of the crystalline implants, although it was suggested that carbachol may have non-specific action on non-cholinergic cells (Kent, 1972).

A related point concerns the physiological mechanism by which intracerebrally administered compounds resulted in the suppression of estrogen/progesterone-activated sexual receptivity. The suppression of lordotic behavior following administration of norepinephrine (NE) (Part C) may have been the result of NE-induced vasoconstriction (Myers, 1974). Regardless of the etiology of the

drug-induced inhibition of sexual receptivity, the implications of these results are important. For example, testing drugs for inhibition of estrogen/progesterone-activated lordosis is a frequent procedure in many experiments concerned with the neurobiological basis of reproductive behavior. Thus, if the drugs being used are capable of producing vasoconstriction, or other physiological effects unrelated to synaptic transmission, the inhibition of behavior may be due to these effects and not to a direct effect upon a brain region which subserves sexual behavior.

A third point concerns the specificity of the behavioral deficits observed following intracerebral administration of drugs to estrogen/progesterone-treated female rats. In some instances, intracerebral drug treatment may interfere with all forms of active behavior. Inhibition of sexual behavior may result from the general deterioration of the animals' health, lethargy, or the production of competing behavioral responses. For example, it has been observed that systemic administration of high doses of compounds which enhance serotonergic neurotransmission (e.g., p-chloroamphetamine, 5-hydroxytryptophan, fenfluramine, lysergic acid diethylamide, and 5-HT) produce a reflexive syndrome which occurs very rapidly (within 3-5 min). This syndrome consists of reciprocal forepaw treading, lateral head weaving, hindlimb abduction, tremor and rigidity; the onset of these reflexes appears to be a specific reflection of activity at the postsynaptic serotonergic receptor (Grahme-Smith, 1971; Hess & Doepfner, 1961; Jacobs et al., 1978). Thus, although no gross behavioral abnormalities were observed in the present experiments

(Part B, Experiment 2), future experiments should be conducted in order to ascertain whether the 5-HT-induced suppression of sexual receptivity was the result of a decrease in the general activity level or was due to the arresting by serotonin of a specific class of behaviors, i.e., sexual behavior.

A final point involves the dissimilarity between central and peripheral effects of drug administration. For example, as noted in the Introduction, systemic administration of drugs which reduce epinephrine, and alpha-adrenergic receptor activity, or increase noradrenergic neurotransmission, tended to facilitate sexual receptivity; drugs which have the opposite effects inhibited sexual receptivity (Davis & Kohl, 1977; Everitt et al., 1975a, b). However, potentiation of alpha-adrenergic neurotransmission, at least at the level of the MPOA (Experiment 1, Part C), had the opposite behavioral effects; as noted in the discussion (Part C) sequential beta-adrenergic blockade and alpha-adrenergic stimulation facilitated sexual receptivity.

These examples, and the results of the present experiments, open to question the validity of an assumption which underlies systemic pharmacological studies of sexual receptivity; which is, that the influence of a neurotransmitter system will be consistent from one brain area to the next. However, using systemic drug administration, the role(s) of specific brain systems such as the MPOA and the MRF may be obscured. Thus, one of the failures of systemic pharmacological treatments, and the interpretation of the

results, lies in the failure to take into account the spatial organization and the biochemical heterogeneity of the brain.

In summary, a productive approach for further elaborating the role of neurotransmitter mechanisms in the mediation of a complex species-specific behavior, namely, receptivity in the female rat, would be one which involved the critical application of anatomical, neurophysiological, and neuropharmacological methods.

# APPENDICES

# APPENDIX A

## ANIMALS

Adult female rats (Sherman Strain) purchased from Camm suppliers (New Jersey) were used in all experiments. The animals were 75-80 days of age at the time of entry into the laboratory. All animals were housed singly in 22.5 x 30 x 30 cm stainless steel cages in a room maintained on a 14:10 reversed light-dark cycle with lights on at 2300 hr. Food and water (.9% saline for adrenalectomized animals) were available ad lib. Adult male rats (Long-Evans strain, Charles River suppliers) were used throughout all experiments as 'stud' males. These animals were housed 6/cage and maintained in the same animal room, under identical conditions.

# APPENDIX B

## SURGICAL AND HORMONAL TREATMENTS

### B.1. Ovariectomy and Hormone Injections

All females were bilaterally ovariectomized or bilaterally ovariectomized and adrenalectomized under light ether anesthesia at approximately 100 days of age. Since female rats do not show high levels of sexual receptivity following the first administration of estradiol benzoate (17-beta estradiol benzoate, Schering Co.; EB) after ovariectomy (Gerall & Dunlap, 1973), all females were administered EB (1 µg/animal/day) for three days and progesterone (0.5 µg/animal) on day 4 after ovariectomy, but were not tested for sexual receptivity. All hormone injections were administered intramuscularly in 0.1 ml sesame oil.

Beginning one week after ovariectomy or ovariectomy/adrenalectomy, the animals were treated with EB (1 µg/day, for three days) followed by 0.5 mg progesterone on day four. Four to six hours after the progesterone injection each animal was given an initial test for sexual receptivity to determine hormone-responsivity. All experimental females were selected on the basis of their readiness to show sexual receptivity under these laboratory conditions. Females that achieved an LQ (Lordosis Quotient = Londosis Frequency/10 mounts x 100) of 50 or better were retained for intracerebral implantation and further experimental testing.

Following administration of EB and progesterone, approximately 98% of the female rats achieved this criteria.

To ascertain if an intracerebral treatment facilitated sexual receptivity, all females received EB (1 µg/day, for three days), and dexamethasone (125 µg/animal) four hr prior to testing for sexual receptivity; dexamethasone sulfate (a synthetic glucocorticoid) was administered to block ACTH release and possible adrenal activation (Paris et al., 1971). This dosage of EB does not normally induce high levels of sexual receptivity when given without progesterone.

To determine if an intracerebral treatment inhibited sexual receptivity, EB (1 µg/day, for three days) and progesterone (0.5 mg/animal) were administered; all animals were then tested 4-6 hr after progesterone administration. This hormone treatment typically induces high levels of sexual receptivity.

## B-2. Cannulation Procedure

Double-barrel stainless steel cannulae were stereotaxically implanted in those animals meeting the criterion of an LQ or 50 or better on the first post-ovariectomy test. The chronically implanted outer cannulae were constructed from 21 gauge stainless steel tubing. The removable inner cannula (27 gauge) permitted repeated chemical stimulation of specific brain areas. Two outer cannulae were cemented together for bilateral implantation such that the two cannulae were .8-1.0 mm apart when implanted. Each outer cannula was fitted with multiple sets of removable inner cannulae; one set

remained in place between experimental tests, and the others were used to administer the crystalline drugs. These inner cannulae were fitted with a 21 gauge hub 2 mm long, which allowed the cannula to penetrate the brain 1 mm beyond the end of the outer guide cannula. A small piece of intramedic tubing, approximately 5 mm long, was fitted over the 2 mm hub and the top of the guide cannulae, such that when the inner cannulae were inserted a snug, airtight fit was achieved.

After the rat had been pretreated with atropine sulfate (Sigma, 2.5 mg/animal; ip. 20 min prior to surgery), the animal was anesthetized with ether and positioned in a Kopf stereotaxic instrument. The stereotaxic coordinates were taken from Albe-Fessard, Stutinsky and Libouban (1965), and were: AP, 8.2, Lat. .8-1.0, Vert. 3.5 for the MPOA; AP, 1.4, Lat. 1.0, Vert. 2.7, for the MRF: and, AP, 4.5, Lat. 1.0, Vert. 3.0 for the PHA.

With the animal's head level and secured in the stereotaxic instrument, the skin, muscle, and fascia were cut and held aside, exposing the skull. Four stainless steel screws were placed in the skull close to the area where the cannula(e) were to be inserted. After drilling one (for unilateral implants) or two (for bilateral implants; these were equidistant from the midline) holes, the cannula(e) were lowered to the desired depth. At this time dental acrylic was placed around the cannula(e), incorporating the screws; this fastened the implanted cannulae to the skull. All implanted cannulae had insert cannulae in them at the time of implantation.

After the dental acrylic had hardened, the remaining wound was closed and the animal was returned to its cage.

## APPENDIX C

## BEHAVIORAL OBSERVATION AND TESTING PROCEDURES

All behavioral observations began at approximately 1400 hr (2 hr into the dark phase of the cycle). At this time the female was placed in a plexiglass observation chamber (80.5 x 50 x 45 cm) with a sexually vigorous male rat. Immediately prior to experimental testing each male was screened for mounting behavior with a non-experimental female rat. In all experimental tests the male was allowed to mount the female 10 times. If a male failed to mount 10 times within a 4 min period, the male was replaced. Lordosis frequency of the female in response to mounts by the male was expressed as a lordosis quotient (LQ: lordosis frequency/10 mounts x 100). In these experiments only the presence or absence of a lordosis response was noted. The criterion for a lordosis was that the female had to display a momentary rigid arching of the back when mounted by the male. No attempt was made to score the quality or degree of dorsiflexion involved in the lordosis response.

## APPENDIX D

## CHEMICAL STIMULATION TECHNIQUES

In each administration of a crystalline chemical to an individual animal, the insert(s) were removed from the conscious animal and replaced with an identical inner cannula containing the crystalline chemical (e.g., carbachol, bethanechol, serotonin, methysergide, cinanserin, LB-46, norepinephrine, dopamine, progesterone, or cholesterol). The inner cannula(e) were loaded by tapping them 5 times into a thin layer of crystalline chemical spread on a glass plate. This method resulted in the accumulation of 9-25 μg of material (depending on the chemical) in the lumen of the inner cannula.

Pharmacological antagonists (Part B, Experiment 3) and the agonist (Part C, Experiment 1) were infused after two 27 gauge injector cannulae were lowered bilaterally into the anterior hypothalamus via the chronically implanted guide cannulae. Injector cannulae were constructed as outlined above. However, these cannulae were connected by calibrated polyethylene tubing (PE 20) to 1.0 cc syringes set in a Harvard Apparatus infusion/withdrawal pump. The infusion/withdrawal pump was controlled by an automatic timer. The timer and the pump were calibrated so that a .05 μl volume of

infusion solution was bilaterally infused into the hypothalamic sites at a rate of 1 µl/min. All infusions lasted 30 sec.

Pharmacological antagonists and the agonist were prepared daily in pyrogen-free artificial cerebrospinal fluid. The artificial cerebrospinal fluid was also used as the control solution. The solution was derived from the neural electrolytes values and was: $Na^+$, 127.6 mM (7.4 g/l); $K^+$, 2.5 mM (0.1 g/l); $Ca^{2+}$, 1.3 mM (0.14 g/l); $Mg^{2+}$, 1.0 mM (0.19 g/l); and $Cl^-$, 134.5 mM (Myers, 1974). Prior to each infusion, all solutions were passed through a 0.22 µ Swinnex millipore filter. Fifteen sec after the infusion the injection cannulae were removed and the blank indwelling cannulae were replaced.

## APPENDIX E

## HISTOLOGICAL PROCEDURE

At the end of the experimental testing the animals were sacrificed and perfused through the heart with saline followed by a 10% formalin solution. The brains were removed, embedded in paraffin or gelatin, and sectioned at 30 μ. Sections in which the implant tracks were visible were mounted on microscope slides; these slides were then stained with cresyl violet and luxol fastblue, thionine, or neutral red. Implant loci were identified according to the atlases of Konig and Klippel (1963) and Albe-Fessard, Stutinsky and Libouban (1965).

At the conclusion of the experiments in which adrenalectomized females were used, all subjects were sacrificed and laparotomized; their abdominal cavities were inspected for regenerated adrenal tissue. Scores from animals in which adrenal tissue was found were eliminated from the data analysis.

## APPENDIX F

## STATISTICAL PROCEDURES

In each of the experiments of Part A, treatment order was randomized within subjects. Friedman's two-way analysis of variance (Siegel, 1962) was used for determining significance within groups following repeated testing. Lordosis quotient scores were analyzed in two ways: (1) first, all animals with an implant in a specific brain region were included to determine whether a treatment had an effect or not; and (2) all animals were then divided into 'lordosis-positive or lordosis-negative responders.' The criteria for being designated a lordosis-positive responder was that an animal had to achieve an LQ of 30 or greater in at least one of the tests following intrahypothalamic or intrareticular treatment. With the exception of the posterior hypothalamic implants, which were included as control implant sites, there were very few negative sites, and separating animals on the basis of this arbitrary criterion of being positive or negative did not have a significant effect upon the outcome of the statistical tests. Thus, significance levels refer to groups containing both positive and negative test scores. Following the determination of statistical significance using a Friedman's analysis of variance, a sign test (Siegal, 1962) was used to determine significance levels between the means of specific

post-intracerebral treatment tests. All significant values had to achieve at least $p < .05$; p values are all two-tailed. In all tables the values are expressed as means $\pm$ standard error of the mean (SEM).

Data from Part B and C were analyzed using a multivariate analysis of variance with repeated measures on two factors (Winer, 1962). Multiple comparisons between means were made using Scheffés Test (Winer, 1962). All significant values had to achieve at least $p < .05$. Sign tests when used (Part B, Experiment 1) are two-tailed; significant values had to achieve at least $p < .05$.

# REFERENCES

# REFERENCES


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

Ahlenius, S., Engel, J., Eriksson, H., & Sodersten, P. Effects of tetrabenazine on lordosis behavior and on brain monoamines in the female rat. Journal of Neural Transmission, 1972b, 33, 155-62.

Albe-Fessard, D., Stutinsky, F., & Libouhan, S. Atlas Stereotaxique du diencephale du Rat Blanc. Paris 1966.

Allen, E. B., Francis, B. F., Robertson, L. L., Colgate, C. E., Johnston, C. G., Doisey, E. A., Lountz, W. B., & Gibson, H. V. The hormone of the ovarian follicle, its localization and action in test animals and additional points bearing upon the internal secretion of the ovary. American Journal of Anatomy, 1924, 64, 95-115.

Baldretti, G., Arcari, G., & Suchowsky, G. K. Studies on a compound antagonistic to serotonin. Experientia, 1965, 21, 396-7.

Ball, J. Sexual responses in female monkeys after castration and subsequent to estrin administration. Psychological Bulletin, 1936, 33, 811.

Bapna, J., Neff, N. H., & Costa, E. A method for studying norepinephrine and serotonin metabolism in small regions of rat brain. Effect of ovariectomy on amine metabolism in anterior and posterior hypothalamus. Endocrinology, 1971, 89, 1345.

Bard, P. A. Central nervous mechanisms for emotional behavior patterns in animals. Research Publications Association Nervous Mental Disease, 1939, 19, 190-215.

Barraclough, C. A. Steroid regulation of reproductive neuroendocrine processes. In R. O. Greep & E. B. Astwood (Eds.), Handbook of Physiology, vol. 11. Endocrinology. Washington: American Physiological Society, 1960.

Beach, F. A. Importance of progesterone to induction of sexual behavior in castrated female rats. Proceedings Society Experimental Biology Medicine, 1942, 51, 367-71.

Bogdanski, D. F., Weissbach, H., & Udenfriend, S. The distribution of serotonin, 5-hydroxytryptophan decarboxylase, and monoamine oxidase in brain. Journal of Neurochemistry, 1957, 1, 272-8.

Boling, J. L. & Blandau, R. J. The estrogen-progesterone induction of mating response in the female rat. Endocrinology, 1939, 25, 259-64.

Booth, D. A. Mechanism of action of norepinephrine in eliciting eating responses after injections into the rat hypothalamus. Journal of Pharmacology and Experimental Therapeutics, 1968, 160, 336-48.

Brownstein, M. Biogenic amine content of hypothalamic nuclei. In L. D. Grant & W. E. Stumpf (Eds.), Anatomical Neuroendocrinology, International Conference Neurobiology of CNS-Hormone Interactions. Chapel Hill, 1974, 393-6.

Brownstein, M., Kobayashi, R. M., Palkovits, M., & Saavedra, J. M. Choline acetyltransferase levels in diencephalic nuclei of the rat. Journal of Neurochemistry, 1975, 24, 35-8.

Brownstein, M., Palkovits, M., Saavedra, J. M., & Kizer, J. S. Distribution of hypothalamic hormones and neurotransmitters within the diencephalon. In L. Martini & W. F. Ganong (Eds.), Frontiers in Neuroendocrinology, vol. 4, 1976, 1-23.

Brownstein, M., Saavedra, J. M., & Palkovits, M. Norepinephrine and dopamine in the limbic system of the rat. Brain Research, 1974, 79, 431-6.

Clemens, L. G. Regulation of sexual behavior in the rat with intracerebral hormone application. Paper presented at the 79th Annual Convention of the American Psychological Association, Washington, D.C., September, 1972.

Clemens, L. G. Perinatal hormones and the modification of adult behavior. In C. H. Sawyer & R. A. Gorski (Eds.), Steroid Hormones and Brain Function. U.C.L.A. Medical Forum. University of California Press, Los Angeles, California, 1972.

Clemens, L. G. Neural plasticity and feminine sexual behavior in the rat. In T. McGill, D. Dewsbury, & B. Sachs (Eds.), Sex and Behavior. Plenum Press, 1977, In Press.

Clemens, L. G., Shryne, J., & Gorski, R. A. Androgen and development of progesterone responsiveness in male and female rats. Physiology and Behavior, 1970, 5, 673-8.

Dahlstrom, A. & Fuxe, K. Evidence for the existence of monoamine containing neurons in the cell bodies of brain stem neurons. Acta Physiologica Scandinavica, 1964, 62, 232, 1-55.

Davidson, J. M. Hormones and Reproductive Behavior. In H. Bolin & S. Glasser (Eds.), Reproductive Biology, Amsterdam; Excerpta Medica, 1972.

Davidson, J. M., Rodgers, C. H., Smith, E. R., & Bloch, G. J. Stimulation of female sex behavior in adrenalectomized rats with estrogen alone. Endocrinology, 1968, 82, 193-5.

Davidson, J. M. & Levine, S. Progesterone and heterotypical sexual behavior in male rats. Journal of Endocrinology, 1969, 44, 129-30.

Davis, G. A. & Kohl, R. The influence of alpha-receptors or lordosis in the female rat. Pharmacology, Biochemistry, and Behavior, 1977, 6, 43-53.

Dempsey, E., Hertz, R., & Young, W. C. The experimental induction of estrous (sexual receptivity) in the normal and ovariectomized guinea pig. American Journal of Physiology, 1936, 116, 201-9.

Dombro, R. S. & Wooley, D. W. Cinnamides as structural analogs and antagonists of serotonin. Biochemical Pharmacology, 1964, 13, 569-76.

Dorner, G., Docke, F., & Hinz, G. Homo- and hypersexuality in rats with hypothalamic lesions. Neuroendocrinology, 1969, 4, 20-4.

Eisenfield, A. J. & Axelrod, J. Selectivity of estrogen distribution in tissues. Journal of Pharmacology and Experimental Therapeutics, 1963, 150, 469-75.

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

Espino, C., Sano, M., & Wade, G. N. Alpha-methyltryptamine blocks facilitation of lordosis by progesterone in spayed estrogen-primed rats. Pharmacology, Biochemistry and Behavior, 1975, 3, 557-9.

Eterovic, A. A. & Bennet, E. L. Nicotinic cholinergic receptor in brain detected by binding of a -($^3$H) bungarotoxin. Biochemistry Biophysica Act, 1974, 362, 346-55.

Everitt, B. J., Fuxe, K., & Hokfelt, T. Inhibitory role of dopamine and 5-hydroxytryptamine in the sexual behavior of female rats. European Journal of Pharmacology, 1974, 29, 187-191.

Everitt, B. J., Fuxe, K., & Hokfelt, T. Serotonin, catecholomines, and sexual receptivity of female rats: Pharmacological findings. Journal Pharmacology (Paris), 1975a, 6, 269-76.

Everitt, B. J., Fuxe, K., Hokfelt, T., & Jonsson, G. Role of monoamines in the control by hormones of sexual receptivity in the female rat. Journal of Comparative Physiological Psychology, 1975b, 89, 556-72.

Feder, H. H., Resko, J. A., & Goy, R. W. Progesterone levels in the arterial plasma of pre-ovulatory and ovariectomized rats. Journal of Endocrinology, 1968, 41, 563-9.

Feder, H. H. & Ruf, K. B. Stimulation of progesterone release and estrous behavior by ACTH in ovariectomized rodents. Endocrinology, 1969, 84, 171-4.

Fonnum, F., Walaas, I., & Iversen, E. Localization of gabaergic, cholinergic and aminergic structures in the mesolimbic system. Journal of Neurochemistry, 1977, 29, 221-30.

Frank, A. H. & Fraps, R. M. Induction of estrus in the ovariectomized golden hamster. Endocrinology, 1945, 37, 357-61.

Fuxe, K. Evidence for the existence of monoamine neurons in the central nervous system. IV. Distribution of monoamine nerve terminals in the CNS. Acta Physiologica Scandinavica, 1965, 64, Supplement 247, 39-85.

Fuxe, K. & Hokfelt, T. Further evidence for the existence of tuberoinfundibular dopamine neurons. Acta Physiologica Scandinavica, 1966, 66, 245-246.

Fuxe, K., Everitt, B. J., & Hokfelt, T. Enhancement of sexual behavior in the female rat by nicotine. Pharmacology, Biochemistry and Behavior, 1977, 7, 147-151.

Fuxe, K. & Jonsson, G. Further mapping of central 5-HT neurons. Studies with the neurotoxic dihydroxytryptamines. Symp. 5-Hydroxytryptamine and other Indolealkylamines in Brain, Caliari 1973.

Gerall, A. A. & Dunlap, J. L. The effect of experience and hormones on the initial receptivity in female and male rats. Physiology and Behavior, 1973, 10, 851-854.

Giudicelli, J. F., Schmitt, H., & Boissier, J. R. Studies on dl-4-4-(2-hydroxy-3-isopropylaminopropoxy)-indole (LB-46), a new potent adrenergic blocking drug. Journal of Pharmacology and Experimental Therapeutics, 1969, 168, 116-126.

Goodman, L. S. & Gilman, A. The Pharmacological Basis of Therapeutics. (4th ed.) New York: The Macmillan Co. 1970.

Grahme-Smith, D. G. Studies in vivo on the relationship between brain tryptophan brain 5-HT synthesis and hyperactivity in rats with a monoamine oxidase inhibitor and l-tryptophan. Journal of Neurochemistry, 1971, 18, 1053-1056.

Greengrass, P. M. & Tonge, S. R. Effects of estrogen and progesterone on brain monoamines: interactions with psychotrophic drugs. Journal of Pharmacy and Pharmacology, 1975, 24, Supplement 1, 121-134.

Grimm, Y. & Reichlin, S. Thyrotropin-releasing hormone (TRH): neurotransmitter regulation of secretion by mouse hypothalamic tissue, in vitro. Endocrinology, 1973, 93, 626-631.

Grossman, S. P. Eating or drinking elicited by direct adrenergic or cholinergic stimulation of hypothalamus. Science, 1960, 132, 301-2.

Grossman, S. P. Behavioral and electroencephalographic effects of microinjections of neurohumors into the midbrain reticular formation. Physiology and Behavior, 1968, 3, 777-786.

Gyermak, L. 5-HT antagonists. Pharmacological Reviews, 1961, 13, 399-39.

Hackman, E., Wirz-Justice, A., & Lichsteiner, M. The uptake of dopamine and serotonin during progesterone decline. Psychopharmacologica (Berlin), 1973, 32, 183.

Haigler, H. J. & Aghajanian, G. K. Peripheral serotonin antagonists: failure to antagonize serotonin brain areas receiving input from prominent serotonergic input. Journal of Neural Transmission, 1974, 35, 257-73.

Hansult, C. D., Uphouse, L., Schlesinger, K., & Wilson, J. R. Induction of estrus in mice: Hypophyseal-adrenal effects. 1972, Hormones and Behavior, 3, 113-12.

Harris, G. W. & Michael, R. P. Hypothalamic mechamisms and the control of sexual behavior in the female cat. Journal of Physiology, 1958, 142, 26-54.

Harris, G. W. & Michael, R. P. The activation of sexual behavior by hypothalamic implants of estrogen. Journal of Physiology, 1964, 171, 275-284.

Hattori, T., McGeer, E. G., Singh, V. K., & McGeer, P. L. Cholinergic synapses of the interpeduncular nucleus. Experimental Neurology, 1977, 55, 666-79.

Herndon, J. G. & Neil, D. B. Amphetamine reversal of sexual impairment following anterior hypothalamic lesions in female rats. Pharmacology, Biochemistry, and Behavior, 1973, 1, 285-288.

Herndon, J. G., Caggiula, A. R., Sharp, D., Ellis, D., & Redgate. Selective enhancement of the lordotic component of female sexual behavior in rats following destruction of central catecholomine-containing systems. Brain Research, 1978, 141, 137-142.

Hess, S. M. & Doepfner, W. Behavioral effects and brain amine content in rats. Archives Internationale Pharmacodynamie, 1961, 134, 89-99.

Holzbauer, M. & Youdim, M. B. H. The estrous cycle and monoamine oxidase activity. British Journal of Pharmacology, 1973, 48, 600-28.

Jacobowitz, D. M. & Goldberg, A. M. Determination of Ach in discrete regions of the rat brain. Brain Research, 1977, 122, 575-577.

Jacobs, B. L., Mosko, S. S., & Trulson, M. G. The investigation of the role of serotonin in mammalian behavior. In R. R. Drucker-Colin and J. L. McGaugh (Eds.), Neurobiology of sleep and memory, Academic Press, New York, In Press, 1978.

Jensen, E. V. & Jacobson, H. I. Basic guides to the mechanisms of estrogen action. Recent Progress Hormone Research, 1971, 181, 567-75.

Kamberi, A. I. & Kobayashi, Y. Monoamine oxidase activity in the hypothalamus and various parts of the brain in some endocrine glands of the rat. Endocrinology, 1970, 93, 356-61.

Karczman, A. G. Neurophysiological, behavioral and neurochemical correlates of the central cholinergic synapses. In O. Vinar, Z. Votava, & P. B. Bradley (Eds.), Advanced in Neuropsychopharmacology, 1971, North-Holland Publ. Co. Amsterdam.

Kataoka, K., Nakamura, Y., & Hassler, R. Habenulo-interpeduncular tract: a possible cholinergic neuron in the rat brain. Brain Research, 1973, 62, 264-267.

Kennedy, G. C. Hypothalamic control of the endocrinological and behavioral changes associated with the estrous cycle in the rat. Journal of Physiology, 1964, 172, 383-92.

Kent, E. W. Behavior of central nervous system single units in the vicinity of topically applied crystalline neurohumors. Physiology and Behavior, 1972, 8, 987-991.

Kobayashi, R. M., Brownstein, M. J., Saavedra, J. M., & Palkovits, M. Choline acetyltransferase content in discrete regions of the rat brainstem. Journal of Neurochemistry, 1975, 24, 637-640.

Kobayashi, F., Kobayashi, T., Kato, J., & Miniquichi, H. Cholinergic and adrenergic mechanisms in female rat hypothalamus with reference to feedback of ovarian steroid hormones. In G. Pincus, T. Nakao, & T. Tait (Eds.), Steroid Dynamics, 1966, New York, Academic Press.

Koe, B. K. & Weissman, A. P-cholorophenylalanine, a specific depletor of brain serotonin. Journal of Pharmacology and Experimental Therapeutics, 1966, 154, 499-516.

Konig, J. F. R. & Klippel, R. A. The Rat Brain. Krieger Huntington, New York, 1970.

Kordon, C. & Glowinski, J. Role of hypothalamic monoanimergic neurons in the gonadotrophin release regulating mechanism. Neuropharmacology, 1972, 11, 153-162.

Krieger, D. Y. & Risso, F. Serotonin mediation of circadian periodicity of plasma 17-hydroxycorticosteroids. American Journal of Physiology, 1969, 217, 1703-1717.

Kuhar, M. J., DeHaven, R. N., Yamamura, H. I., Romelspacher, H., & Simon, J. R. Further evidence for cholinergic habenulo-interpeduncular neurons: Pharmacological and functional characteristics. Brain Research, 1975, 97, 265-275.

Lake, N. Studies of the habenulo-interpeduncular pathyway in rats. Experimental Neurology, 1973, 41, 113-132.

Law, T. & Meagher, W. Hypothalamic lesions and sexual behavior in the female rat. Science, 1958, 128, 1626.

Leibowitz, S. F. Reciprocal hunger-regulating circuits involving alpha- and beta-adrenergic receptors located respectively in the ventro-medial and lateral hypothalamus. Proceeding National Academy of Science, 1970, 67, 1063-1070.

Leibowitz, S. F. Paraventricular nucleus: a primary site mediating adrenergic stimulation of feeding and drinking. Pharmacology, Biochemistry and Behavior, 1978, 8, 163-177.

Lewis, P. R. & Lewis, C. C. D. The cholinergic limbic system: projections to hippocampal formation, medial cortex, nuclei of the ascending cholinergic reticular formation and the subfornical organ and supraoptic crest. Brain, 1967, 90, 521-40.

Lindstrom, L. H. The effect of pilocarpine in combination with MAOI, imipramine or desmethylimipramine on oestrous behavior in female rats. Psychopharmacologia, 1970, 17, 160-168.

Lindstrom, L. H. The effect of pilocarpine in female rats after treatment with monoamine depletors on monoamine synthesis inhibitors. European Journal of Pharmacology, 1971, 15, 60-65.

Lindstrom, L. H. Further studies on cholinergic mechanisms and hormone-activated copulatory behavior in the female rat. Journal of Endocrinology, 1973, 56, 275-283.

Lindstrom, L. H. & Meyerson, B. J. The effect of pilocarpine, oxotremorine and arecolin in combination with methylatropine on hormone activated oestrous behavior in ovariectomized rats. Psychopharmacologia, 1967, 11, 405-413.

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

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

Luttge, W. G., Wallis, C. J., & Hall, N. R. Effects of pre- and post-treatment with unlabelled steroids on the in-vivo uptake of $^{3}H$-progestin in selected brain regions, uterus, and plasma of the female mouse brain. Brain Research, 1974, 71, 105-115.

McGeer, P. L., McGeer, E. G., Singh, V. K., & Chase, W. H. Choline acetyltransferase localization in the central nervous system by immunohistochemistry. Brain Research, 1974, 81, 373-379.

Meyerson, B. J. Central nervous monoamines and hormone induced estrous behavior in the spayed rat. Acta Physiologica Scandinavica, 1964a, 248, 5-32.

Meyerson, B. J. Estrus behavior in spayed rats after estrogen or progesterone treatment in combination with reserpine or tetrabenazine. Psychopharmacologia, 1964b, 6, 210-218.

Meyerson, B. J. The effect of neuropharmacological agents on hormone-activated estrus behavior in ovariectomized rat. Archive Internationales de Pharmacodynamie et de Therpie, 1964c, 150, 4-33.

Meyerson, B. J. The effects of imipramine and related antidepressant drugs on estrus behavior in ovarietomized rats activated by progesterone, reserpine or tetrabenazine in combination with estrogen. Acta Physiologica Scandinavica, 1966a, 67, 411-422.

Meyerson, B. J. Oestrous behavior in estrogen treated ovariectomized rats after chloropromazine alone or in combination with progesterone, tetrabenazine or reserpine. Acta Pharmacologica et Toxicologica, 1966b, 24, 363-367.

Meyerson, B. J. Relationship between the anesthetic and gestagenic action and estrous behavior-inducing activity of different progestins. Endocrinology, 1967, 81, 369-374.

Meyerson, B. J. Amphetamine and 5-hydroxytrptamine inhibition of copulatory behavior in the female rat. Annales Medicinae Experimentalis et Biologiae Fenniae, 1968, 46, 394-398.

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

Morley, B. J., Lorden, J. F., Brown, G. B., Kemp, G. E., & Bradley, R. J. Regional distribution of nicotinic ACh receptors in rat brain. Brain Research, 1977, 134, 161-164.

Myers, R. D. & Haksh, T. L. Control of body temperature in the unanesthetized monkey by cholinergic and animergic systems in the hypothalamus. Journal of Physiology, 1969, 202, 483-500.

Myers, R. D. Methods for perfusing different structures of the brain. In R. D. Myers (Ed.), Methods in Psychobiology, 1972, 2, Academic Press, New York.

Myers, R. D. Handbook of Drug and Chemical Stimulation of the Brain. Behavioral Pharmacological and Physiological Aspects. Van Nostrand Reinhold, New York, 1974.

Nance, D. M., Shryne, J., & Gorski, R. A. Septal lesion: Effects on lordosis behavior and pattern of gonadotrophin release. Hormones and Behavior, 1974, 5, 73-81.

Nance, D. M., Shryne, J., & Gorski, R. A. Effects of septal lesion on behavioral sensitivity of female rats to gonadal hormones. Hormones and Behavior, 1975a, 6, 59-64.

Nance, D. M., Shryne, J., & Gorski, R. A. Facilitation of female sexual behavior in male rats by septal lesions: An interaction with estrogen. Hormones and Behavior, 1975b, 6, 289-299.

Nance, D. M., McGinnis, M., & Gorski, R. A. Interaction of olfactory and amygdala destruction with septal lesions: effects on lordosis behavior. Neuroscience Abstracts, 6th Annual Meeting, Society for Neuroscience, 653, 1976.

Palka, Y. S. & Sawyer, C. H. The effects of hypothalamic implants of ovarian steroids on oestrous behavior in rabbits. Journal of Physiology, 1966, 211, 225-228.

Palkovits, M. & Jacobowitz, D. M. Topographic atlas of catecholamines and acetylcholinesterase containing neurons in the rat brain. II. Hindbrain (Mesencephalon and Rhombencephalon). Journal of Comparative Neurology, 1974, 157, 14-29.

Palkovits, M., Saavedra, J. M., Kobayashi, R. M., & Brownstein, M. Choline acetyltransferase content of limbic nuclei of the rat. Brain Research, 1974, 79, 443-450.

Paris, C. A., Resko, J. A., & Goy, R. W. A possible mechanism for induction of lordosis by reserpine in spayed rats. Biology of Reproduction, 1971, 4, 23-30.

Passonen, M. K., MaClean, P. D., & Giarman, N. J. 5-hydroxy-tryptamine (serotonin; enteramine) content of structures in the limbic system. Journal of Neurochemistry, 1975, 1, 326-333.

Pfaff, D. W. Luteinizing hormone-releasing factor potentiates lordosis behavior in hypophysectomized, ovariectomized female rats. Science, 1973, 182, 1148-1149.

Pfaff, D. W. & Keiner, M. Atlas of estradiol concentrating cells in the central nervous system of the female rat. Journal Comparative Neurology, 1973, 151, 121-158.

Polz-Tejera, G. J., Schmidt, T., & Karton, A. J. Autoradiographic localization of alpha-bungarotoxin-binding sites in the central nervous system. Nature, 1975, 258, 349-351.

Powers, B. J. & Valenstein, E. S. Sexual receptivity: facilitation by medial preoptic lesions in female rats. Science, 1972, 178, 1003-1005.

Proudfit, H. K. & Anderson, E. G. Blockade by serotonin antagonists of brainstem evoked potentials recorded from dorsal and ventral roots. Federation Proceedings, 1973, 32, 303.

Robson, J. M. Induction of oestrous changes in the monkey and bitch by triphenyl ethylene. Proceeding Society Experimental Biology and Medicine, 1938, 38, 153-157.

Rodgers, C. & Schwartz, N. B. Differentiation between neural and hormonal control of sexual behavior and ganadotrophin secretion in the female rat. Endocrinology, 1976, 98, 778-786.

Rosendorf, C. & Cranston, W. I. Effects of intrahypothalamic and intraventricular NE and 5-HT on hypothalamic blood flow in the conscious rabbit. Circulation Research, 1971, 28, 492-502.

Ross, J., Claybaugh, C., Clemens, L. G., & Gorski, R. A. Short latency induction of estrous behavior with intracerebral gonadal hormones in ovariectomized rats. Endocrinology, 1971, 89, 32-38.

Routenberg, A. The effects of chemical stimulation in dorsal mid-brain tegmentum on self-stimulation in hypothalamus and septal area. Psychonomic Science, 1965, 3, 41-42.

Rudy, T. A. & Wolf, H. A. The effect of intrahypothalamically injected sympathomimetic amines on temperature regulation in the cat. Journal Pharmacology and Experimental Therapeutics, 1971, 179, 218-235.

Russek, M., Morgenson, G. J., & Stevenson, J. A. F. Calorigenic, hyperglycemic and anorexigenic effects of adrenaline and noradrenaline. Physiology and Behavior, 1967, 2, 428-433.

Saavedra, J. M. Localization of Biogenic Amine-synthesizing enzymes in discrete hypothalamic nuclei. In L. D. Grant & W. E. Stumpf (Eds.), Anatomical Neuroendocrinology. International Conference Neurobiology of CNS-Interactions, Chapel Hill, 1974.

Saavedra, J. M., Palkovits, M., Brownstein, M. J., & Axelrod, J. Distribution in the nuclei of the rat hypothalamus and preoptic region. Brain Research, 1974, 77, 157-165.

Saavedra, J. M. & Zivin, J. Tyrosine hydroxylase and dopamine-β-hydroxylase: Distribution in discrete areas of the rat limbic system. Brain Research, 1976, 105, 517-524.

Schaepdryver, A. F., Preziosi, P., & Scapagnini, U. Brain monoamines and stimulation of ACTH release. Archives International Pharmacodynamics, 1969, 180, 11-18.

Schlecter, M. D. & Rosecrans, J. A. Behavioral evidence for two types of cholinergic receptors in the CNS. European Journal of Pharmacology, 1971, 15, 375-378.

Schleiffer, L. S. & Eldefrawi, M. E. Identification of the nicotinic and muscarinic acetylcholine receptors in the subcellular fractions of mouse brains. Neuropharmacology, 1974, 13, 53-63.

Schneider, H. P. G. & McCann, S. M. Mono- and indolamines and the control of LH secretions. Endocrinology, 1970, 86, 1127-1133.

Shute, C. C. D. & Lewis, P. R. The ascending cholinergic reticular system; Neocortical olfactory and subcortical projections. Brain, 1967, 90, 497-520.

Siegel, S. Nonparametric statistics for the behavioral sciences. New York: McGraw Hill Book Co., 1956.

Singer, J. J. Hypothalamic control of male and female sexual behavior in female rats. Journal of Comparative Physiological Psychology, 1968, 66, 738-742.

Sturpf, W. E. Estradiol-concentrating neurons in the periventricular neurons. American Journal of Anatomy, 1970, 129, 207-218.

Ungerstedt, U. Stereotaxic mapping of the monoamine pathways in the rat brain. Acta Physiologica Scandinavica, 1971, 367, 1-48.

Uphouse, L. L., Wilson, J. R., & Schlesinger, K. Induction of estrous in mice a possible role of adrenal progesterone. Hormones and Behavior, 1970, 1, 255-264.

Wade, G. N., Harding, C. F., & Feder, H. H. Neural uptake of (1,2-3H) progesterone in ovariectomized rats, guinea pigs and hamsters: correlation with species differences in behavioral responsiveness. Brain Research, 1973, 61, 357-361.

Ward, I. L., Crowley, W. R., Zemlen, F. P., & Margules, D. L. Monoaminergic mediation of female sexual behavior. Journal of Comparative Physiological Psychology, 1975, 88, 53-61.

Whalen, R. E. & Luttge, W. G. Differential localization of progesterone uptake in the brain. Role of sex, estrogen pretreatment and adrenalectomy. Brain Research, 1971, 33, 144-155, a.

Whalen, R. E. & Luttge, W. G. Role of the adrenal in the preferential accumulation of progestin by mesencephalic structures. Steroids, 1971, 18, 141-145 b.

Winer, B. J. Statistical Principles in Experimental Design. New York: McGraw Hill Book Co., 1971.

Yamamura, H. I., Kuhar, M. J., & Snyder, S. H. In vivo identification of muscarinic cholinergic receptor binding in rat brain. Brain Research, 1974, 80, 170-176.

Young, W. C. The Mamalian Ovary. In W. C. Young (Ed.), Sex and Internal Secretions. William and Wilkins, Co., Baltimore, 1173-1239, vol. 1961.

Zemlen, F. P., Ward, K. L., Crowley, W. R., & Margules, D. L. Activation of lordotic responding in female rats by suppression of serotonergic activity. Science, 1973, 179, 1010-1011.

Zemlen, F. P., Trulson, M. E., Howell, R., & Hoebel, B. G. Influence of p-chloroamphetamine on sexual reflexes and brain monoamines levels. Brain Research, 1977, 123, 347-356.

Zschack, L. L. & Wurtman, R. J. Brain $^{3}$H-catechol synthesis and the vaginal estrus cycle. Neuroendocrinology, 1973, 11, 144-167.