This is to certify that the

dissertation entitled

CHOLINERGIC REGULATION
OF FEMALE SEXUAL BEHAVIOR

presented by

GARY PETER DOHANICH

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Zoology

Lynwood Clemens
Major professor

Date August 11, 1981

CHOLINERGIC REGULATION OF FEMALE SEXUAL BEHAVIOR

By

Gary Peter Dohanich

A DISSERTATION

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

DOCTOR OF PHILOSOPHY

Department of Zoology

1981

ABSTRACT

CHOLINERGIC REGULATION OF
FEMALE SEXUAL BEHAVIOR

By

Gary Peter Dohanich

The role of cholinergic activity in the regulation of hormone-dependent sexual behavior in female rats was examined. In two experiments, stimulation of cholinergic receptors in specific areas of the brain facilitated the occurrence of sexual behavior. Specifically, carbachol, a cholinergic receptor agonist, increased the incidence of lordosis in estrogen-primed female rats following bilateral microinfusion (0.5 ug/side) into the medial preoptic area or ventromedial hypothalamus. The behavioral response was normally highest 15 min after carbachol infusion and had returned to control levels by 90 min. Intracerebral carbachol facilitated lordosis at lower levels of estrogen priming than did systemic progesterone which may indicate that carbachol and progesterone achieve their effects by different mechanisms. The ineffectiveness of exogenous progesterone at these low levels of estrogen priming also suggests that carbachol infusion did not facilitate lordosis by inducing the release of adrenal progestins. Carbachol failed to activate lordosis following infusion into the mesencephalic reticular formation or frontal cortex. It appears that the potential of a brain area to respond to cholinergic stimulation may be related to the sensitivity

of that area to estrogen.

In two other experiments, an agent which interferes with central cholinergic activity was found to inhibit sexual behavior displayed by female rats. Bilateral forebrain infusion of hemicholinium-3 (HC-3, 1.25, 5, or 7.5 ug/side), an acetylcholine synthesis blocker, reduced the incidence of lordosis in females receiving behaviorally-active combinations of estrogen and progesterone. The inhibition of lordosis by HC-3 was prevented when choline chloride (120 ug/side) or carbachol (0.5 ug/side) was simultaneously infused with HC-3. There was no evidence of behavioral inhibition following infusion of HC-3 into the frontal cortex of sexually receptive females.

In the final experiment, the ability of estrogen to alter <u>in vitro</u> binding of a radioactive cholinergic muscarinic antagonist within certain areas of the brain was demonstrated. However, these data did not consistently support behavioral findings since an expected increase in muscarinic binding was not detected at the estrogen-priming dose (0.125 ug) utilized in behavioral experiments. Furthermore, higher doses of estrogen (1 or 10 ug) increased the number of muscarinic binding sites in the medial basal hypothalamus but decreased the number of muscarinic bindig sites in the medial preoptic area.

These experiments suggest that lordosis in female rats is influenced by an estrogen-dependent cholinergic

mechanism within the brain. The facilitation of lordosis by cholinergic stimulation of specific brain regions does not appear to be correlated with an estrogen-induced increase in cholinergic binding as measured by *in vitro* uptake of a muscarinic antagonist.

"I never been nobody's idol
But at least I got a title."

- Merle Haggard

ACKNOWLEDGEMENTS

The contributions of various individuals were indispensable to the completion of this work. The result is dedicated to those individuals: Dr. Lynwood Clemens for his insight, enthusiasm, and confidence; Jeffrey Witcher for his invaluable collaboration; David Brigham for his capable technical assistance; Cheryl Rapp for her skillful histology and illustrations; and Jo Ann Dohanich for her proficient typing and patience.

The advice and guidance of Drs. Edward Convey, Richard Rech, and John King are gratefully acknowledged.

TABLE OF CONTENTS

Table of Contents cont'd

LIST OF TABLES

LIST OF FIGURES

List of Figures cont'd

INTRODUCTION

Various behaviors which increase the probability of successful mating have evolved in vertebrates. Lordosis is a distinctive posture assumed by females of several mammalian species during the period of sexual receptivity. It is characterized by flexion of the epaxial muscles which causes a ventral arching of the spine and elevation of the perineum (Pfaff, 1980). In species such as the rat, in which the male achieves multiple intromissions before ejaculating, lordosis promotes genital stimulation of both sexes and facilitates sperm deposition during ejaculation. The lordosis response is elicited in the female rat by the mount of a male and is dependent on somatosensory stimulation to the flanks, rump, and tail base (Pfaff, 1980).

Steroid hormones secreted by the ovary are clearly the principle hormonal factors controlling lordosis (Young, 1961). Accordingly, removal of the ovaries abolishes the capacity for sexual behavior in female rats. Sexual behavior is restored in ovariectomized females by systemic administration of estrogen (estradiol) followed by progesterone (Boling and Blandau, 1939). This treatment regimen mimics the natural hormonal profile of the intact cyclic female such that maximum receptivity is preceded by several days of rising estrogen titers followed by a

brief increase in progesterone levels (Clemens and Christensen, 1975). However, when given in sufficient quantities, estradiol alone will activate a high incidence of lordosis in ovariectomized female rats (Pfaff, 1970).

Although lordosis has been described as a stereotyped reflex (Pfaff, 1980), the neuroendocrine mechanisms which regulate the occurrence of this behavior are complex. The selective uptake of estradiol from the circulation by specific areas of the brain appears to be prerequisite. Visualization of tritiated estradiol uptake by autoradiography has identified the medial preoptic area (POA) and the ventromedial hypothalamus (VMH) as two forebrain areas with dense distributions of neurons which concentrate estradiol (Pfaff and Keiner, 1973). The ability of preoptic and hypothalamic neurons to take up estradiol suggests that these particular cells may direct the performance of an estrogen-dependent behavior such as lordosis.

Appropriately, results obtained with a variety of techniques have implicated both the POA and the VMH in the control of estrogen-activated lordosis. For example, estradiol benzoate (EB) has been reported to activate sexual receptivity in ovariectomized rats within several days after direct implantation in either the POA or the VMH (Lisk, 1962; Lisk and Barfield, 1975; Yanase and Gorski, 1976; Barfield and Chen, 1977). The VMH appears to be particularly sensitive to estrogen stimulation since a higher incidence of lordosis was observed in female rats

following implantation of EB in the VMH than was observed following implantation of EB in the POA (Barfield and Chen, 1977). Furthermore, dilute estradiol implants confined to the VMH activated lordosis in female rats when combined with systemic administration of progesterone (Rubin and Barfield, 1980). However, this same treatment was ineffective when dilute estradiol implants were placed in other brain areas including the POA.

In addition to displaying different sensitivities to estrogen, evidence suggests that the POA and VMH may exert different actions in mediating female sexual behavior. Although lesions of the VMH have been demonstrated to inhibit lordosis in female rats treated with estrogen (Mathews and Edwards, 1977), lesions of the POA increased the potential of estrogen-treated females to display lordosis (Powers and Valenstein, 1972). Further, electrical stimulation of the VMH facilitated lordosis (Pfaff and Sakuma, 1979) while stimulation of the POA has been found to inhibit lordosis (Malsbury and Pfaff, 1973). Finally, EB has been shown to increase the number of cells in the VMH displaying detectable spontaneous activity while decreasing their number in the POA (Bueno and Pfaff, 1976). These results seem to suggest that the VMH exerts an excitatory influence over estrogen-activated lordosis and the POA exerts a tonic inhibitory influence.

In contrast to the POA and VMH, implantation of EB in the mesencephalic reticular formation (MRF) failed to

facilitate lordosis in female rats (Yanase and Gorski, 1976). However, combinations of EB implants in the MRF with systemically injected progesterone or progesterone implants in the MRF with systemically injected EB activated a high incidence of lordosis (Ross, Claybaugh, Clemens, and Gorski, 1971; Yanase and Gorski, 1976). Furthermore, local exposure to estrogen was necessary for MRF implants of progesterone to be effective (Yanase and Gorski, 1976). Although the MRF does not concentrate tritiated estradiol as proficiently as basal forebrain areas (Pfaff and Keiner, 1973) mesencephalic control of female sexual behavior nevertheless may be mediated by an interaction between estrogen and progesterone.

The sequence of events initiated by intracellular uptake of estradiol and culminating in the lordosis response is presently speculative. However, evidence (O'Malley and Means, 1974; Woo and O'Malley, 1975) that ovarian hormones regulate the synthesis of specific proteins within the chick oviduct by interaction with the cellular genome may have important implications for the hormonal control of sexual behavior. A hormone, such as estrogen, is believed to diffuse passively into the cytoplasm of a target cell from the general circulation and bind to high-affinity receptor proteins. These hormone-receptor complexes are subsequently translocated into the cell nucleus where they interact with nonhistone chromosomal proteins. This interaction facilitates transcription

of new messenger RNA, with the location of the nonhistone protein on the DNA molecule determining the code sequence of the messenger RNA synthesized. Consequently, these events may result in ribosomal formation of various proteins which regulate cellular function and composition.

The process by which ovarian hormones influence synthesis of proteins in the chick oviduct is clearly applicable to estrogen target cells in the brain. It has been suggested that exposure of appropriate brain areas to estrogen may alter the activity of various putative neurotransmitter systems, thereby activating the neural circuitry which mediates female sexual behavior (McEwen, Davis, Parsons, and Pfaff, 1979). Conceivably, the synthesis of a wide variety of transmitter-related proteins may be influenced by the presence of estrogen within a cell. For instance, estrogen might effect an alteration in neurotransmitter activity by regulating the production of enzymes which are responsible for the synthesis or degradation of a particular neurotransmitter. Alternatively, estrogen might control the synthesis or degradation of membrane-bound neurotransmitter receptor proteins, modify the formation of enzymes involved in secondary cyclase systems, or determine the level of various intracellular transport proteins.

Although the specific mechanism or mechanisms have yet to be identified, evidence supports the conclusion that the activation of lordosis by estrogen is dependent

upon protein synthesis. Actinomycin D, an inhibitor of DNA-dependent RNA synthesis, blocked estrogen-progesterone-induced lordosis in female rats when applied to the POA, VMH, or third ventricle within 12 hr after estrogen treatment (Quadagno, Shryne, and Gorski, 1971; Terkel, Shryne, and Gorski, 1973; Ho, Quadagno, Cooke, and Gorski, 1973). Cyclohexamide, an inhibitor of protein synthesis, produced an identical effect on lordosis following infusion into the POA or third ventricle (Quadagno and Ho, 1975). More recently, systemic administration of anisomycin, a protein synthesis inhibitor with low toxicity, was reported to block estrogen-progesterone-activated lordosis, as well as induction of a specific protein, the progestin receptor, in the POA and VMH (Rainbow, Davis, and McEwen, 1980). These results indicate that some form of estrogen-induced protein synthesis within preoptic and hypothalamic neurons is critical to the occurrence of lordosis.

If estrogen activates lordosis in female rats by altering the activity of certain neurotransmitters within the brain, then pharmacological manipulations which produce similar alterations in neurotransmitter activity should also activate lordosis. Meyerson (1964) first demonstrated that systemic administration of agents which depleted the level of whole brain serotonin facilitated lordosis in female rats. However, these antiserotonergic compounds were only effective in ovariectomized females that had been

pretreated with low doses of estrogen. Since the original work of Meyerson, every known major neurotransmitter system has been shown to influence the display of sexual behavior in female rodents in some manner. Consistently, the ability of any pharmacological agent to increase the incidence of lordosis in female rodents has been contingent upon estrogen pretreatment. The importance of neurotransmitter functions to sexual behavior now appears certain and the potential of estrogen to regulate the activity of these neurotransmitter systems is a logical mechanism of hormone action. However, at present, it is not possible to completely circumvent the role of estrogen by pharmacologically altering the activity of neurotransmitters which mediate lordosis. This observation suggests that estrogen probably influences a number of cellular functions which are critical to lordosis.

The initial finding that pharmacological depletion of brain serotonin facilitated lordosis in female rats primed with estrogen (Meyerson, 1964) was later confirmed and extended by several investigators. Increases in the frequency of lordosis were reported in estrogen-treated female rats following administration of a serotonin synthesis inhibitor, parachlorophenylalanine (PCPA); serotonin receptor blockers, methysergide and cinanserin; or serotonin release and reuptake blockers, reserpine and tetrabenazine (Meyerson and LeWander, 1970; Zemlan, Ward, Crowley, and Margules, 1973; Everitt, Fuxe, and Hokfelt,

1974; Everitt, Fuxe, Hokfelt, and Jonsson, 1975; Ward, Crowley, Zemlan, and Margules, 1975; Eliasson and Meyerson, 1977; Foreman and Moss, 1978a). In addition, reductions in the incidence of lordosis were observed in sexually receptive female rats following treatment with agents which enhance central serotonin activity such as monoamine oxidase inhibitors, lysergic acid diethylamide, 5-hydroxytryptophan, fenfluramine, or serotonin (Meyerson, 1966; Eliasson, Michanek, and Meyerson, 1972; Everitt et al., 1974, 1975; Meyerson, Carrer, and Eliasson, 1974; Eliasson, 1976; Eliasson and Meyerson, 1976, 1977; Foreman and Moss, 1978a). Furthermore, the POA, VMH, anterior hypothalamus, posterior hypothalamus, and medial forebrain bundle have been identified as active sites in this inhibitory serotonergic system (Ward et al., 1975; Foreman and Moss, 1978a).

Despite this impressive body of evidence, reservations have been raised concerning the validity of serotonergic inhibition of lordosis. It has been suggested that increases in the incidence of lordosis following treatment with antiserotonergic agents may be due to a drug-induced release of adrenal steroids, particularly progesterone (reviewed by Clemens, 1978). In support of this contention, PCPA failed to facilitate lordosis in adrenalectomized, estrogen-primed rats (Eriksson and Sodersten, 1973). Reserpine was similarly ineffective in adrenalectomized, estrogen-primed mice (Uphouse, Wilson, and

Schlesinger, 1970). Furthermore, an increased concentration of plasma progesterone was detected in female rats following reserpine treatment (Paris, Resko, and Goy, 1971). When the rise in plasma progesterone was prevented by pretreatment with a corticotropin suppressor, reserpine failed to facilitate lordosis in estrogen-primed females. In contrast, other reports have demonstrated that the activation of lordosis by PCPA or methysergide treatment persisted in absence of the adrenal glands (Zemlan et al., 1973) although the magnitude of response may be reduced (Everitt et al., 1975).

Since PCPA was found to deplete brain catecholamines, as well as brain serotonin, the roles of dopamine and norepinephrine in the mediation of lordosis have also received considerable attention. Indeed, the increase in the frequency of lordosis observed following PCPA treatment was reported to be temporally correlated with a reduction in brain catecholamine content rather than with a reduction in brain serotonin level (Ahlenius, Engel, Eriksson, Modigh, and Sodersten, 1972). However, much of the subsequent research on the effects of the catecholamines on female sexual behavior has proven to be ambiguous and contradictory. Some of this confusion appears to stem from the multiple receptor types which are believed to mediate catecholamine transmission.

Support for an inhibitory dopaminergic system was provided by a series of pharmacological experiments.

Systemic administration of alpha methylparatyrosine, a catecholamine synthesis inhibitor, or pimozide, a dopamine receptor blocker, facilitated lordosis in female rats primed with estrogen (Ahlenius et al., 1972; Everitt et al., 1974, 1975; Davis and Kohl, 1977; Fuxe, Everitt, and Hokfelt, 1977). Adrenalectomy failed to reduce this response to dopamine antagonists (Everitt et al., 1974, 1975). In addition, dopamine receptor agonists, such as apomorphine and ET 495, were found to reduce the frequency of lordosis in females brought into receptivity by administration of estrogen and progesterone (Everitt et al., 1974, 1975; Eliasson, 1976). Pretreatment with pimozide prevented the reduction in lordosis induced by apomorphine, further implicating a dopaminergic system (Meyerson, Carrer, and Eliasson, 1974; Eliasson and Meyerson, 1976). An inhibition of lordosis following d-amphetamine treatment was also reported to be blocked by pimozide pretreatment (Michanek and Meyerson, 1977). Furthermore, selective depletion of brain catecholamines by intraventricular administration of a neurotoxin, 6-hydroxydopamine, increased the incidence and duration of lordosis in female rats primed with estrogen (Herndon, Caggiula, Sharp, Ellis, and Redgate, 1978; Caggiula, Herndon, Scanlon, Greenstone, Bradshaw, and Sharp, 1979).

Although these results suggest a significant inhibitory role for a central dopamine system, several experiments directly contradict this conclusion. For example,

in contrast to the purported inhibitory effects of dopamine receptor agonists, a facilitation of lordosis has also been reported in hormone-primed female rats following systemic administration of apomorphine or ET 495 (Hamburger-Bar and Rigter, 1975; Everitt and Fuxe, 1977). This facilitation was not mediated by release of adrenal steroids (Hamburger-Bar and Rigter, 1975). These contradictory effects of dopamine receptor agonists on lordosis may be related to drug dose. While high doses of agonists inhibit lordosis in estrogen-progesterone-treated females, low doses appear to facilitate lordosis in estrogen-primed females. Everitt and Fuxe (1977) have attempted to reconcile these effects with the concept of an inhibitory dopaminergic system. They suggest that, at high doses, dopamine agonists act at postsynaptic receptors to inhibit lordosis; whereas, at low doses, these agonists act at presynaptic receptors to reduce endogenous dopamine release and thereby facilitate lordosis. Although appealing, this hypothesis has yet to be substantiated.

A facilitative role for dopamine has also been indicated by infusion of dopaminergic agents into various brain areas. Microinfusion of dopamine or apomorphine into the POA or VMH was found to increase the incidence of lordosis in hormone-primed female rats (Foreman and Moss, 1979; Dohanich and Clemens, 1980). Conversely, the frequency of lordosis was reduced in females treated with high doses of estrogen following preoptic or hypothalamic

infusion of dopamine receptor blockers, haloperidol and alpha flupenthixol (Foreman and Moss, 1979). However, since the maximum behavioral response to dopamine infusion usually was observed 1-2 hr after infusion, it is difficult to interpret this effect as a direct neurotransmitter action. The contribution of adrenal steroids, although not assessed, may represent a significant factor in these intracerebral infusion experiments.

As in the case of dopamine, the role of norepinephrine in the control of hormone-dependent lordosis has not been well defined. A facilitative influence of the noradrenergic system was initially indicated by the observation that d-amphetamine potentiated the stimulatory effect of a dopamine receptor blocker, pimozide, on lordosis (Everitt et al., 1975). It was suggested that this enhancement was due to an amphetamine-induced release of norepinephrine. More recently, infusion of norepinephrine into the POA or VMH was reported to increase the incidence of lordosis in estrogen-primed female rats (Foreman and Moss, 1978b). Selective stimulation of beta noradrenergic receptors in the POA or VMH by infusion of isoproterenol similarly facilitated lordosis while blocking beta receptors by infusion of propranolol inhibited lordosis (Foreman and Moss, 1978b). However, the possible dependence of noradrenergic facilitation of lordosis on adrenal steroid release has not been determined.

Several experiments have also indicated an inhibitory

role for norepinephrine. Generally, systemic or intracerebral administration of alpha noradrenergic receptor blockers such as piperoxane, yohimbine, phentolamine, or phenoxybenzamine has been found to increase the frequency of lordosis in estrogen-primed females (Everitt et al., 1975; Foreman and Moss, 1978b). In addition, lordosis was inhibited in receptive females following treatment with alpha noradrenergic receptor agonists, clonidine and methoxamine (Davis and Kohl, 1977; Foreman and Moss, 1978b). The inhibition of lordosis by clonidine was prevented by pretreatment with the alpha receptor antagonist, yohimbine, but not by the alpha receptor antagonist, phenoxybenzamine. Davis and Kohl (1977) suggested, therefore, that noradrenergic alpha receptors may be present in two forms with unequal potential to inhibit lordosis.

Consequently, in the rat, available data seem to indicate a facilitative role for beta noradrenergic receptors and an inhibitory role for alpha noradrenergic receptors in the mediation of hormone-induced lordosis. Alternatively, norepinephrine appears to exert a major facilitative action on lordosis in the guinea pig based on observations that agents which promote noradrenergic activity, such as clonidine, increased lordosis duration while agents which reduce noradrenergic activity, such as phenoxybenzamine or a norepinephrine synthesis blocker, inhibited the display of lordosis (Crowley, Feder, and Morin, 1976; Crowley, Nock, and Feder, 1978; Nock and

Feder, 1979).

Only one report presently available documents the role of the gamma-aminobutyric acid (GABA) system in the control of lordosis (McGinnis, Gordon, and Gorski, 1980). Picrotoxin, a GABA receptor blocker, was found to decrease the incidence of lordosis in estrogen-progesterone-treated female rats following bilateral infusion into the substantia nigra. In addition, the frequency of lordosis was increased in estrogen-primed females by bilateral nigral infusion of hydrazinopropionic acid (HPA), an agent which reduces GABA degradation. The maximum behavioral response occurred 2 hr after HPA infusion. The authors suggest that increased nigral GABA facilitated lordosis by inhibiting dopamine activity in the striatum and nucleus accumbens. However, the delayed onset of an HPA effect and the failure to control for HPA-induced release of adrenal steroids weaken their conclusion. Furthermore, although evidence is presented that dopamine activity was modified in the striatum and nucleus accumbens following nigral infusion of GABAminergic agents, a proposed inhibitory effect of dopamine on lordosis is itself tenuous.

The role of each of the major neurotransmitter systems reviewed thus far may be summarized. A substantial amount of evidence strongly indicates that serotonin exerts an inhibitory action on lordosis. The facilitation of lordosis following treatment with certain agents which reduce serotonergic activity may be the result of adrenal

steroid secretion. However, the ability of several anti-serotonergic compounds to activate lordosis in adrenalectomized females clearly implies a central action of serotonin. The contribution of other neurotransmitter systems to the control of lordosis is not as well characterized. Dopamine activity has been reported to exert either facilitative or inhibitory actions on lordosis depending on the experimental contingency. Agonists, as well as antagonists, increased the incidence of lordosis in estrogen-primed female rats even following adrenalectomy. The suggestion that these contradictory effects may be mediated by two types of dopamine receptors, presynaptic and post-synaptic, has yet to be confirmed experimentally. Similarly, both facilitative and inhibitory actions have been described for the norepinephrine system. It has been hypothesized that stimulation of beta receptors activates lordosis, whereas, stimulation of alpha receptors inhibits lordosis. However, the available evidence is weak, particularly since a facilitation of lordosis by beta agonists has not been demonstrated in adrenalectomized females. Finally, recent data indicate a facilitative role of brain GABA in the control of lordosis. Again, the possible contribution of adrenal steroids has not been determined. Furthermore, the proposed mediation of the facilitative effect of GABA by the dopamine system must be regarded as speculative.

The remaining major neurotransmitter, acetylcholine,

may represent another central influence on hormone-activated lordosis. Lindstrom and Meyerson (1967) reported that systemic administration of cholinergic receptor agonists such as pilocarpine, oxotremorine, or arecoline reduced the incidence of lordosis in female rats primed with estrogen and progesterone. This inhibition was observed within 30 min after injection and was prevented by pretreatment with atropine, a cholinergic receptor blocker. Systemic pretreatment with methylatropine, an agent which only blocks cholinergic receptors in the periphery, failed to prevent the reduction in lordosis by cholinergic agonists, implying a central action of these compounds.

Lindstrom (1970) later found that the inhibition of lordosis following pilocarpine treatment was magnified when females were pretreated with monoamine oxidase inhibitors, pargyline or nialamide. Monoamine oxidase inhibitors were previously shown to inhibit lordosis in receptive females, presumably by increasing central monoaminergic, principally serotonergic activity (Meyerson, 1964). Lindstrom suggested, therefore, that elevations in cholinergic and monoaminergic activity may inhibit lordosis by related mechanisms. Subsequently, it was demonstrated that pretreatment with the monoamine synthesis inhibitor PCPA prevented the inhibition of lordosis by pilocarpine (Lindstrom, 1971). Furthermore, pretreatment with a catecholamine synthesis inhibitor failed to block the inhibitory effects of pilocarpine. Lindstrom concluded

that pilocarpine inhibited lordosis in estrogen-progesterone-treated females via a serotonergic mechanism.

Pilocarpine and oxotremorine were also reported to increase the incidence of lordosis in female rats primed with estrogen (Lindstrom, 1973). In contrast to the short latency of the inhibitory effects previously observed (30 min), the facilitation of lordosis by cholinergic agonists occurred 4 hrs after systemic injection. The increase was blocked by atropine pretreatment, reinforcing a cholinergic interpretation. However, cholinergic agonists failed to facilitate lordosis in estrogen-primed females that had been adrenalectomized, hypophysectomized, or pretreated with a corticotropin suppressor. Consequently, it appears that the enhancement of lordosis following pilocarpine or oxotremorine treatment was dependent on a drug-induced release of adrenal steroids.

The work of Lindstrom suggests that the inhibitory effect of cholinergic agonists is mediated by a serotonergic mechanism while the facilitative effect of cholinergic agonists is mediated by adrenal steroids. The cholinergic compounds used by Lindstrom act primarily at cholinergic muscarinic receptors. A more recent study (Fuxe et al., 1977), indicated that stimulation of cholinergic nicotinic receptors following systemic administration of nicotine increased the incidence of lordosis in estrogen-primed female rats. This facilitation was observed within 5 min after injection and was blocked by

pretreatment with a nicotinic receptor blocker but not by pretreatment with catecholaminergic agents. Although the effect of nicotine treatment on lordosis was not evaluated in adrenalectomized females, it is doubtful that adrenal steroids would activate lordosis within 5 min after release.

Rodgers and Law (1968) implanted a muscarinic receptor agonist, carbachol, or a muscarinic receptor blocker, atropine, in various brain areas and reported a facilitation of lordosis in ovariectomized female rats. The failure to prime females with estrogen and the negligible level of response to intracerebral treatments make the significance of their results difficult to interpret. However, evidence has recently been presented which strongly suggests that stimulation of central cholinergic muscarinic receptors facilitates lordosis independently of adrenal secretions, contradicting the early reports of Lindstrom. Implantation of muscarinic receptor agonists carbachol and bethanechol in the POA or MRF consistently activated lordosis in female rats primed with estrogen (Clemens, Humphrys, and Dohanich, 1980). This facilitation was usually observed within 30 min of intracerebral treatment, persisted in the absence of the adrenal glands, and was blocked by pretreatment with the muscarinic receptor blocker atropine. In support of a facilitative role of muscarinic receptors, Singer (1968) earlier reported that systemic administration of a high dose of atropine alone

reduced the incidence of lordosis in female rats treated with estrogen and progesterone.

The experiments comprising this dissertation were designed to extend the analysis of cholinergic facilitation of sexual receptivity in female rats. Several design features were introduced in order to more fully characterize this phenomenon. The contradictory behavioral effects often observed following treatment with various pharmacological agents may arise partly from the mode of administration. The systemic injection technique often exposes the entire brain, if not the entire body of an organism, to the drug. Nonphysiological responses under these experimental conditions may be expected. Consequently, in order to limit drug actions to specific areas of the brain, cholinergic agents were applied directly to brain sites implicated in the control of female sexual behavior. In addition, compounds were administered in solution form via a microinfusion technique which delivers low doses of a drug and provides transient stimulation of specific brain areas not possible with traditional intracerebral implants of crystalline compounds. The effects of a cholinergic agonist, carbachol, as well as a cholinergic antagonist, hemicholinium-3, were investigated. Since cholinergic mediation of lordosis appears to be a hormone-dependent phenomenon, the behavioral responses of female rats following intracerebral cholinergic treatment were examined under a variety of estrogen and estrogen-

progesterone regimens. Finally, the ability of manipulations of the cholinergic system to influence sexual behavior may indicate that ovarian homones alter cholinergic processes within the brain. In the last experiment, the effect of estrogen treatment on a cholinergic parameter, specifically muscarinic receptor binding, was evaluated in specific areas of the female rat brain.

GENERAL METHOD
(Experiments 1-4)

Sherman female rats, 60-70 days of age, were obtained from Camm Research Company, Wayne, New Jersey. The animals were housed singly with free access to food and water. A reversed light-dark cycle was maintained in the vivarium (14 hr light: 10 hr dark, light off at 1100 hr).

Females were placed under ether anesthesia (Mallinckrodt, Inc.) or Ketamine anesthesia (Parke, Davis, and Co.) and bilaterally ovariectomized at 75-85 days of age. To verify a normal behavioral response to exogenous hormone treatment, all ovariectomized females were pretested for lordosis prior to stereotaxic surgery. Females were injected intramuscularly with 0.5 ug estradiol benzoate (EB, Schering Corp.) 72, 48, and 24 hr before behavioral testing. Approximately 5 hr before testing 0.5 mg progesterone (Sigma Chemical Co.) was injected intramuscularly. EB and progesterone were administered in 0.1 ml volumes of sesame seed oil (Sigma Chemical Co.). Hormone doses always represent the total dose administered to each animal per day.

Behavioral tests were conducted in a Plexiglas arena (45 x 50 x 58 cm) occupied by a Long-Evans stimulus male (Charles River Laboratories). Each female was mounted a

total of 10 times during a single test. Any female failing to receive 10 mounts within 10 min was transferred to another arena with a different male where the test was completed. The number of lordosis responses observed per 10 mounts was recorded and a lordosis quotient was computed for each female: LQ = (number of lordoses/10 mounts) x 100.

Females displaying an LQ of 70 or higher on the pretest underwent stereotaxic surgery under ether or Ketamine anesthesia approximately 1 wk later. Cholinergic drugs were infused intracerebrally via a double-barrel cannula assembly constructed from stainless-steel hypodermic tubing (Small Parts, Inc.). Each assembly consisted of a chronic 23 gauge outer barrel (guide) and a removable 27 gauge inner barrel (insert). Guides were implanted bilaterally 1 mm dorsal to the target brain sites and anchored to the skull with machine screws and dental acrylic. Each insert extended 1 mm beyond the end of the guide into the brain site. Stereotaxic coordinates were provided by Albe-Fessard, Stutinsky, and Libouban (1966) for five different sites as follows: POA (anterior 7.9, lateral 1.0, horizontal 4.0); VMH (anterior 5.8, lateral 0.8, horizontal 2.0); MRF (anterior 3.2, lateral 1.0, horizontal 2.0); stria terminalis (anterior 7.9, lateral 1.0, horizontal 5.5); frontal cortex (anterior 10.0, lateral 1.0, horizontal 9.0).

One wk after stereotaxic surgery all females were injected with EB 72, 48, and 24 hr before behavioral testing. The dose of EB administered varied with experiment. In some experiments, 0.5 mg progesterone or 0.1 ml sesame seed oil was injected intramuscularly 4-5 hr before behavioral testing. Prior to intracerebral infusion, the insert was removed and replaced by a 28 gauge infusion insert which extended into the target site. The infusion insert was connected by PE-20 polyethylene tubing to a syringe mounted on a reciprocal microinfusion pump (Harvard Apparatus). A 0.5 ul volume of solution was delivered over 30 sec sequentially through each cannula (1 ul/animal). Following infusion, the infusion insert was replaced by the original insert. For intracerebral infusions, cholinergic compounds were dissolved in an artificial cerebrospinal fluid (CSF) containing 130 mM NaCl, 25 mM Na HCO_3, 5 mM Na_2H PO_4, 30 mM KCL, 8 mM Mg Cl_2, and 13 mM $CaCl_2$ (pH 6.8). The agents administered included carbamylcholine chloride (carbachol, Sigma Chemical Co.), hemicholinium-3 (HC-3, Aldrich Chemical Co.), and choline chloride (Sigma Chemical Co.). During the test session each female was pretested for lordosis and, immediately after, was bilaterally infused with the appropriate drug or vehicle solution. The effects of intracerebral treatment on lordosis were tested at various times after infusion during 1-3 weekly sessions.

After the final behavioral test, all females were

anesthetized with ether or phenobarbital and perfused intracardially with 0.9% saline followed by 10% buffered formalin. Brains were removed and frozed coronal brain sections (50 u) were taken. Implant locations were verified following neutral red staining.

Data were analyzed with parametric analyses of variance usually followed by Newman-Keuls range tests (Winer, 1971) or with nonparametric Kruskall-Wallis analyses of variance, Mann-Whitney U-tests, Wilcoxon matched pairs T tests, or Fisher exact probability tests (Siegel, 1956). Log transformations were made where appropriate.

EXPERIMENT 1. Facilitation of Lordosis in Hormone-treated Female Rats Following Intracerebral Infusion of Carbachol

Intracerebral implants of cholinergic agonists in crystalline form have been found to increase the incidence of lordosis in female rats primed for 3 days with 1 ug EB (Clemens et al., 1980). The facilitation of lordosis occurred 30-60 min after intracerebral treatment and persisted up to 120 min. Unfortunately, the crystalline implant technique delivers a high, and often inexact, dose of a drug (approximately 10 ug) to the brain site, activating a behavioral response which may be sustained for hours. Under these conditions, the effects of repeated behavioral tests and adrenal steroid release may contribute to the prolonged behavioral response often observed. In Experiment 1, the ability of carbachol, a cholinergic receptor agonist, to facilitate lordosis in female rats was investigated using a microinfusion technique which provides precise control of drug dose and limits the duration of drug stimulation. This procedure should elicit a reliable, prompt, and transient facilitation of behavior.

The effect of carbachol on lordosis was evaluated under a variety of hormone priming conditions. Systemic administration of progesterone is known to activate

lordosis in female rats treated with EB (Boling and Blandau, 1939). Since progesterone, as well as cholinergic agonists, facilitate lordosis in estrogen-primed females, a common mechanism may underlie their actions. In Experiment 1, the behavioral effects of progesterone, administered by systemic injection, and carbachol, administered by intracerebral infusion, were compared. Female rats were treated with either EB and progesterone, EB and carbachol, or EB, progesterone, and carbachol. The various doses of EB selected (0.13, 0.17, and 0.25 ug for 3 days) activate only a low to moderate incidence of lordosis, even when combined with progesterone treatment. The doses of progesterone (0.5 mg) and carbachol (0.5 ug/cannula) activate a high incidence of lordosis when combined with adequate amounts of estrogen. Utilizing a series of low EB doses, in combination with optimum doses of progesterone and/or carbachol, allowed comparison of the behavioral effects of progesterone and carbachol, as well as assessing the summation of their actions.

METHOD

Ovariectomized female rats were bilaterally implanted with cannulae in the POA according to the procedure outlined in the General Method section (See Figure 1). Implanted females received 3 weekly tests beginning 1 wk after stereotaxic surgery. All females were primed with 0.13 ug EB at 72, 48, and 24 hr prior to the first weekly

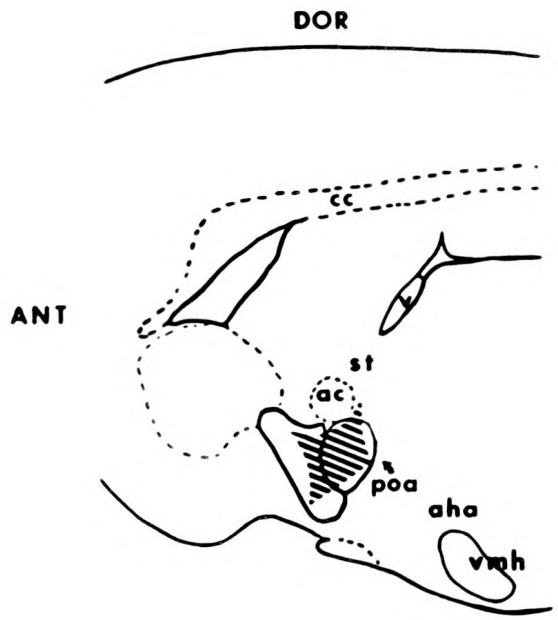


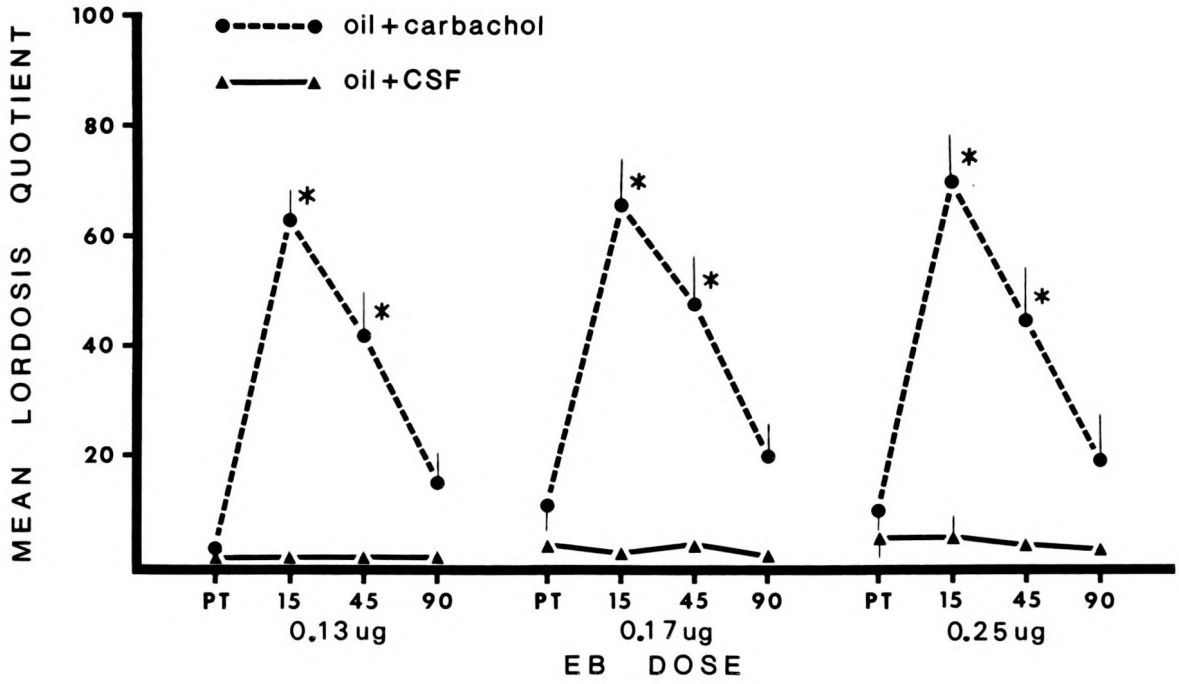
Figure 1. Distribution of cannula tips as verified histologically in female rats from Experiment 1. Only animals with implants in the areas indicated were included in data analysis. The plate represents a sagittal section takem 1.0 mm lateral to the midline. Adapted from Albe-Fessard et al. (1966). Abbreviations: ANT, anterior; DOR, dorsal; ac, anterior commissure; aha, anterior hypothalamic area; cc, corpus callosum; poa, preoptic area; st, stria terminalis; v, ventricle; vmh, ventromedial hypothalamus.

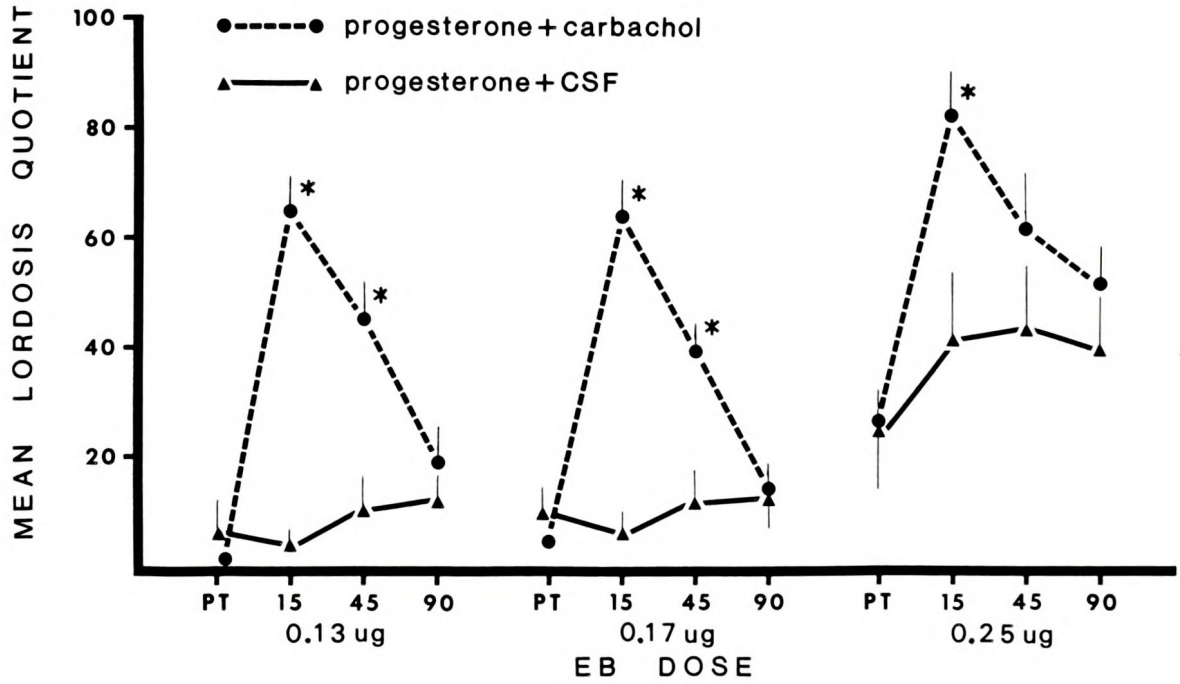
test. The priming dose of EB was increased to 0.17 ug for the second weekly test and 0.25 ug for the third weekly test. Approximately 4-5 hr before each test females were injected with either 0.5 mg progesterone or 0.1 ml sesame oil. During a test session, each female was pretested for lordosis and afterward bilaterally infused with either carbachol (0.5 ug/cannula) or CSF vehicle. The effects of intracerebral treatments on lordosis were tested 15, 45, and 90 min after infusion. Each female received the same combination of carbachol or CSF with progesterone or oil over the 3 wks of testing.

RESULTS

Significant increases in LQ were observed following preoptic infusion of carbachol (0.5 ug/cannula) under all hormone regimens (Figure 2). At the 0.13 ug dose of EB, analysis of variance revealed significant main effects of intracerebral treatment ($p < .001$) and time after infusion ($p < .001$), as well as a significant treatment x time interaction ($p < .001$). Females primed with EB and oil displayed a higher incidence of lordosis 15 min ($p < .05$, Newman-Keuls test) and 45 min ($p < .05$) after carbachol infusion than after CSF infusion. Equivalent increases in LQ were observed 15 min ($p < .05$) and 45 min ($p < .05$) after carbachol infusion in females primed with EB and progesterone. The incidence of lordosis in both groups receiving carbachol returned to CSF levels by 90 min

Figure 2. Mean lordosis quotients recorded from female rats following bilateral infusion of carbachol (0.5 ug/cannula) or artificial cerebrospinal fluid (CSF) vehicle into the medial preoptic area. Estradiol benzoate (EB) was injected intramuscularly 72, 48, and 24 hr before behavioral testing and the dose was increased each wk for all females. Oil (0.1 ml, upper panel) or progesterone (0.5 mg, lower panel) was injected intramuscularly 4-5 hr before testing. Each female received the same combination of carbachol or CSF with progesterone or oil over the 3 wk of testing. Intracerebral treatment was administered immediately following the preinfusion test (PT). The effect of intracerebral treatment on lordosis was tested 15, 45, and 90 min after infusion. For each treatment combination, n = 10-14. *$p < .05$ (carbachol vs. CSF, Newman-Keuls).


oil + carbachol
oil + CSF
MEAN LORDOSIS QUOTIENT
100
80
60
40
20
*
PT 15 45 90
0.13 ug
PT 15 45 90
0.17 ug
PT 15 45 90
0.25 ug
EB DOSE


progesterone + carbachol
progesterone + CSF
MEAN LORDOSIS QUOTIENT
100
80
60
40
20
*
PT 15 45 90
0.13 ug
PT 15 45 90
0.17 ug
PT 15 45 90
0.25 ug
EB DOSE

after infusion.

Similar increases in LQ following carbachol treatment were evident at the 0.17 ug dose of EB where significant effects of treatment ($p < .001$), time ($p < .001$) and treatment x time interaction ($p < .001$) were detected. Once again females primed with EB, in combination with either oil or progesterone, displayed a higher incidence of lordosis 15 min ($p < .05$) and 45 min ($p < .05$) after carbachol infusion than after CSF infusion. There were no significant treatment differences in behavior by 90 min after infusion.

At the 0.25 ug dose of EB, analysis of variance indicated significant main effects of treatment ($p < .001$) and time ($p < .001$), and a significant treatment x time interaction ($p < .001$). In females primed with EB and oil, the incidence of lordosis was significantly higher 15 min ($p < .05$) and 45 min ($p < .05$) after carbachol infusion than after CSF infusion. As at lower EB doses, LQ returned to the CSF level by 90 min after carbachol infusion. In females primed with 0.25 ug EB and progesterone, the incidence of lordosis was higher after carbachol infusion than after CSF infusion only at 15 min after intracerebral treatment ($p < .05$).

Soliciting behaviors, such as ear wiggling and hopping and darting, were not activated by any treatment combination.

SUMMARY

In Experiment 1, female sexual behavior, indicated by the incidence of lordosis, was significantly increased in estrogen-treated female rats following bilateral infusion of a cholinergic receptor agonist, carbachol (0.5 ug/ cannula), into the POA. Infusion of an artificial CSF vehicle failed to facilitate lordosis. The incidence of lordosis was normally highest 15 min after carbachol infusion, began to wane by 45 min, and had returned to control levels by 90 min. Centrally administered carbachol activated lordosis at lower levels of estrogen priming than did systemically administered progesterone.

EXPERIMENT 2. Brain Regions Implicated in Cholinergic Mediation of Lordosis

A variety of evidence has implicated the POA, VMH, and MRF in the control of hormone-dependent sexual behavior in female rats. However, the ability of intracerebral implants of estrogen to facilitate lordosis appears to vary among these three brain regions. Although estrogen implants in the POA or VMH have both been shown to activate lordosis, the VMH appears to be sensitive to a lower level of estrogen stimulation (Barfield and Chen, 1977; Rubin and Barfield, 1980). Furthermore, estrogen implants in the MRF failed to facilitate lordosis, unless combined with systemically administered progesterone (Yanase and Gorski, 1976). Progesterone implants in the MRF also activated lordosis in females treated systemically with estrogen (Ross et al., 1971; Yanase and Gorski, 1976). Both the POA and VMH concentrate estrogen from the circulation more heavily than the MRF (Pfaff and Keiner, 1973).

In Experiment 2, the incidence of lordosis was assessed in estrogen-primed female rats following microinfusion of the cholinergic receptor agonist, carbachol, into several brain areas including the POA, VMH, and MRF. Differences in the ability to concentrate and respond to estrogen may determine the potential of carbachol to

facilitate lordosis following infusion into these brain regions.

METHOD

Double-barrel cannulae were bilaterally implanted in the POA, VMH, MRF, or frontal cortex of ovariectomized female rats as described in the General Method section. Following a 1 wk recuperative period, females were injected intramuscularly with 0.13 ug EB 72, 48, and 24 hr before behavioral testing. On the day of testing, females were pretested for lordosis and afterward bilaterally infused with either carbachol (0.5 ug/cannula) or CSF vehicle. The effects of intracerebral treatments on lordosis were tested 15, 45, and 90 min after infusion. On the second wk of testing, 0.13 ug EB was again administered intramuscularly to all females 72, 48, and 24 hr before testing and intracerebral treatment (carbachol or CSF) was reversed for each female.

RESULTS

Mean lordosis quotients $\pm$ SEM are presented in Figure 3. For each site, nonparametric comparisons were made between the change in lordosis frequency (postinfusion minus preinfusion) following carbachol versus CSF with each subject serving as its own control (Wilcoxon matched-pairs T test). Significant increases in the incidence of lordosis were observed 15 min ($p < .005$) and 45 min ($p < .005$) after infusion of carbachol into the POA or VMH. The

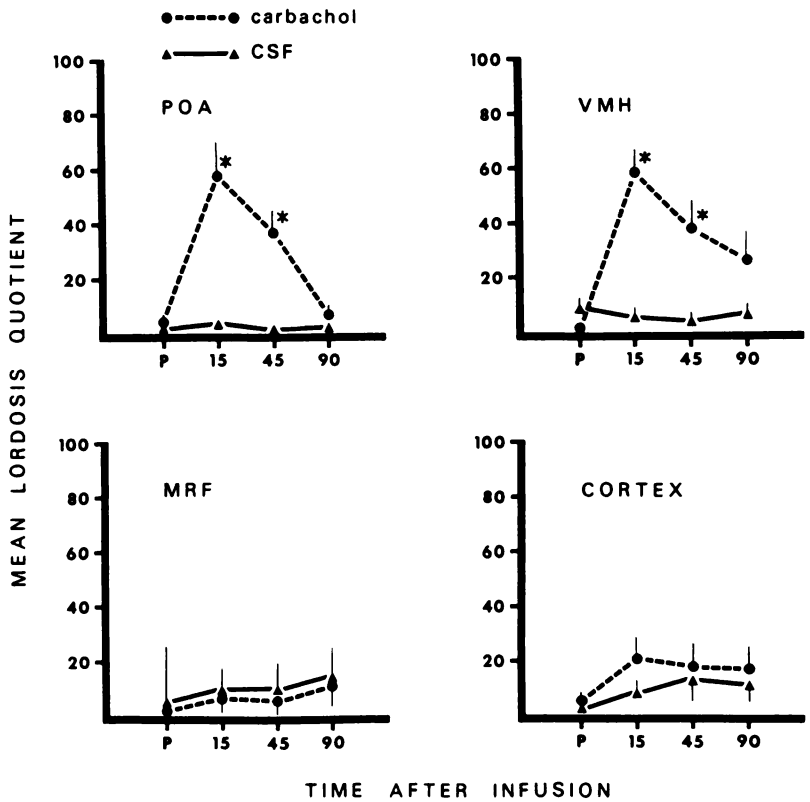


Figure 3. Mean lordosis quotients recorded from female rats following bilateral infusion of carbachol (0.5 ug/cannula) or artificial cerebrospinal fluid (CSF) vehicle into various brain areas. All females were injected with 0.13 ug estradiol benzoate 72, 48, and 24 hr before behavioral testing. Carbachol or CSF was bilaterally infused into the medial preoptic area (POA), ventromedial hypothalamus (VMH), mesencephalic reticular formation (MRF), or frontal cortex. Intracerebral treatment was reversed for each female over 2 wk of testing. Intracerebral treatment was administered immediately following the preinfusion test (P) and behavioral effects were tested 15, 45, and 90 min after infusion. For each site, n = 9-12. *$p < .005$ (carbachol vs. CSF, Wilcoxon T, one-tailed).

frequency of lordosis was not significantly different from CSF levels by 90 min after carbachol infusion in either site. Proceptive behaviors, such as ear wiggling and hopping and darting, were never observed during behavioral tests. Infusion of carbachol into the MRF or cortex failed to significantly facilitate lordosis on all tests.

Location of the insert cannula tips are presented in Figure 4. Infusion sites which resulted in an LQ increase of 40 or more were identified as positive. As indicated in Table 1, Fisher exact probability tests revealed that carbachol induced lordosis in a significantly higher proportion of females than CSF in both the POA ($p < .005$) and VMH ($p < .005$).

SUMMARY

In Experiment 2, the facilitation of lordosis following intracerebral infusion of carbachol was shown to be a site-specific phenomenon. Carbachol increased the incidence of lordosis in estrogen-primed female rats following bilateral infusion (0.5 ug/cannula) into either the POA or the VMH. As in Experiment 1, the behavioral response was highest 15 min after carbachol infusion and returned to control levels within 90 min after infusion. Carbachol failed to activate lordosis following infusion into the MRF or frontal cortex.

Figure 4. Carbachol infusion sites in the medial preoptic area (upper left), ventromedial hypothalamus (upper right), frontal cortex (lower left), and mesencephalic reticular formation (lower right). An increase in lordosis quotient of 40 or more over the pretest recorded on at least 1 postinfusion test was considered a positive effect (positive sites--circles; negative sites--triangles). Histology was unavailable for 1 ventromedial hypothalamic implant. Adapted from Konig and Klippel (1974).

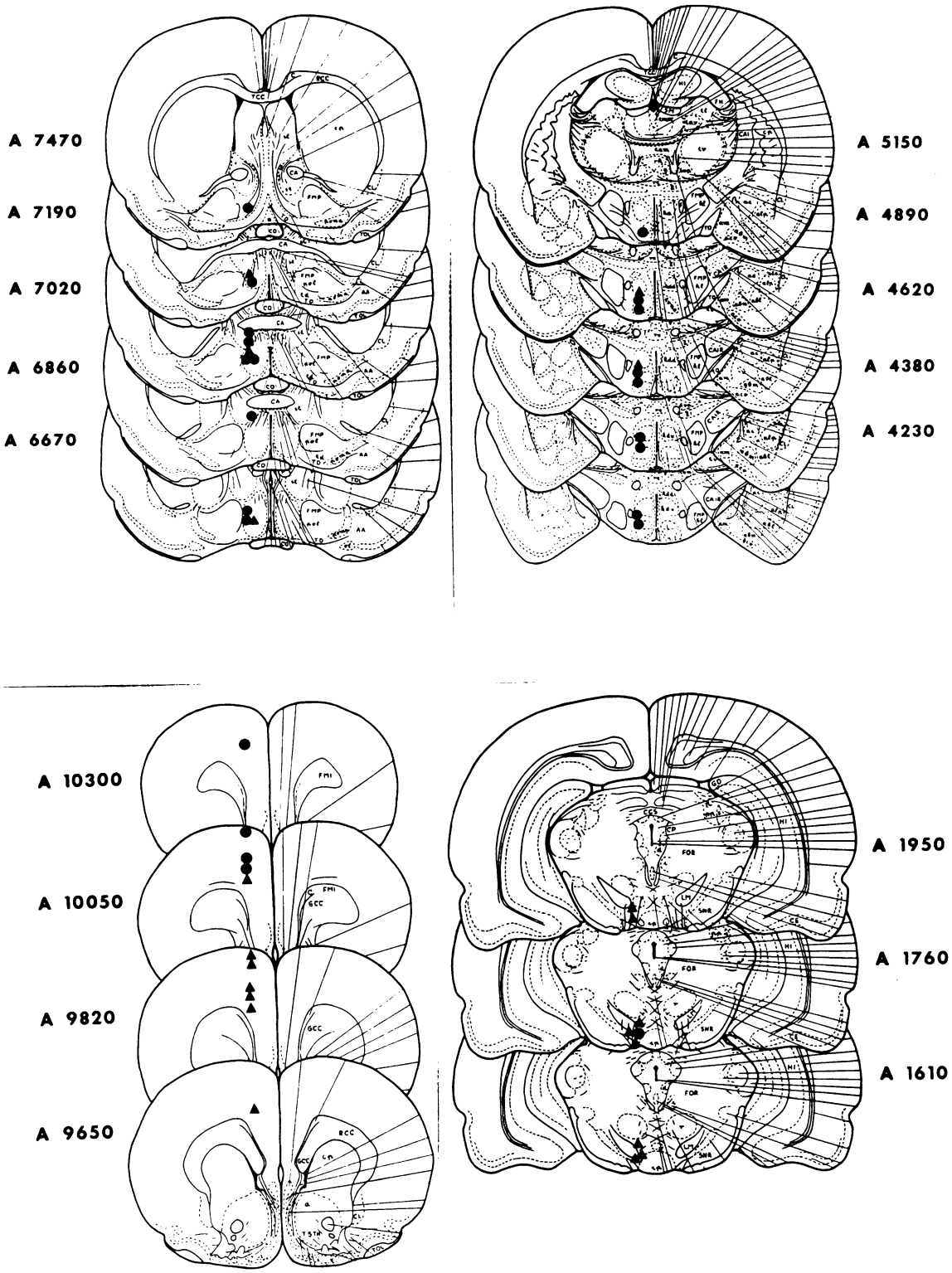

A 7470
A 7190
A 7020
A 6860
A 6670
A 5150
A 4890
A 4620
A 4380
A 4230
A 10300
A 10050
A 9820
A 9650
A 1950
A 1760
A 1610

TABLE 1

Proportion of Female Rats Responding
Following Intracerebral Infusion of Carbachol
or Artificial Cerebrospinal Fluid (CSF) Vehicle

SITE	TREATMENT	PROPORTION RESPONDING[1]
POA	Carbachol	9/12*
	CSF vehicle	0/12
VMH	Carbachol	10/12*
	CSF vehicle	0/12
MRF	Carbachol	1/9
	CSF vehicle	1/9
Cortex	Carbachol	4/11
	CSF vehicle	1/11

[1]An increase in lordosis quotient of 40 or more over the pretest recorded on at least one postinfusion test was considered a positive response.

*$p < .005$ (carbachol vs. CSF, Fisher exact probability test, one-tailed).

EXPERIMENT 3. Inhibition of Lordosis in Hormone-treated Female Rats Following Intracerebral Infusion of Hemicholinium-3

EXPERIMENT 3a

Based on the ability of cholinergic agonists to facilitate lordosis in female rats (Clemens et al., 1980, Experiments 1 and 2), it was predicted that disruption of central cholinergic activity in receptive females would result in a reduction in the incidence of lordosis. In Experiment 3a, the behavioral effects of a cholinergic antagonist, HC-3, were examined in ovariectomized female rats brought into sexual receptivity by administration of estrogen and progesterone. This anticholinergic agent has been shown to retard the synthesis of acetylcholine by competitively inhibiting transport of the precursor, choline, across neuronal membranes (MacIntosh, Birks, and Sastry, 1956; Gardiner, 1961; Guyenet, Lefresne, Rossier, Beaujouan, and Glowinski, 1973). In Experiment 3a, HC-3 was bilaterally infused near the dorsal-medial border of the bed nucleus of the stria terminalis, an area shown to heavily concentrate tritiated estradiol (Pfaff and Keiner, 1973).

METHOD

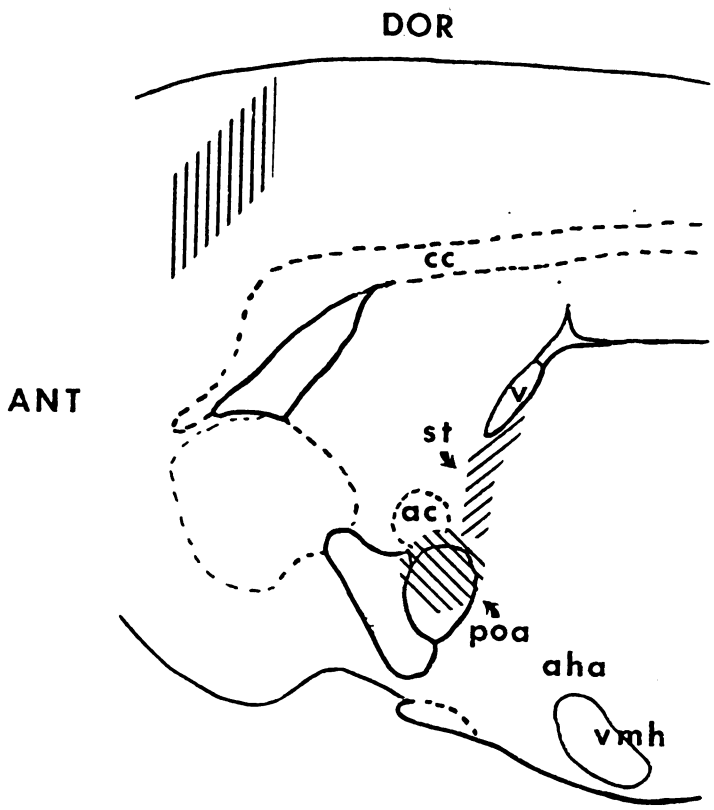
Ovariectomized female rats were bilaterally implanted with cannulae in the stria terminalis according to the

procedure outlined in the General Method section (See Figure 5). During the following 2 wk, all females were injected intramuscularly with 2 ug EB 72, 48, and 24 hr before behavioral testing. Progesterone (0.5 mg) was injected intramuscularly 4-5 hr before testing. During the test session, each female was pretested for lordosis and afterward bilaterally infused with one of three solutions: 7.5 ug HC-3/cannula, 5 ug HC-3/cannula, or CSF vehicle. The effects of intracerebral treatments on lordosis were tested 15 and 30 min after infusion. Each female received the same hormonal and intracerebral treatment on 2 consecutive wk.

RESULTS

Intracerebral infusion of HC-3 (Figure 6) significantly reduced the incidence of lordosis in females treated with EB (2 ug) and progesterone (0.5 mg). The change in LQ following HC-3 infusion (pretest minus test) was compared to the change in LQ observed following CSF infusion at corresponding times after intracerebral treatment (Mann-Whitney U test). During the first wk of treatment, significant reductions in LQ were recorded at 15 and 30 min following infusion of 5 ug HC-3/cannula (at 15 min, $p < .05$; at 30 min, $p < .01$) or 7.5 ug HC-3/cannula (at 15 min, $p < .01$; at 30 min, $p < .01$). During the second wk of treatment, similar reductions in LQ were observed only following infusion of the higher dose of HC-3 (7.5 ug/cannula: at 15 min, $p < .01$; at 30 min, $p < .01$). Infusion

Figure 5. Distribution of cannula tips as verified histologically in female rats from Experiments 3. Only animals with implants in the areas indicated were included in data analysis. The plate represents a sagittal section taken at 1.0 mm lateral to the midline. Adapted from Albe-Fessard et al. (1966). Abbreviations: ANT, anterior; DOR, dorsal; ac, anterior commissure; aha, anterior hypothalamic area; cc, corpus callosum; poa, preoptic area; st, stria terminalis; v, ventricle; vmh, ventromedial hypothalamus.



STRIA TERMINALIS

PREOPTIC AREA

CORTEX

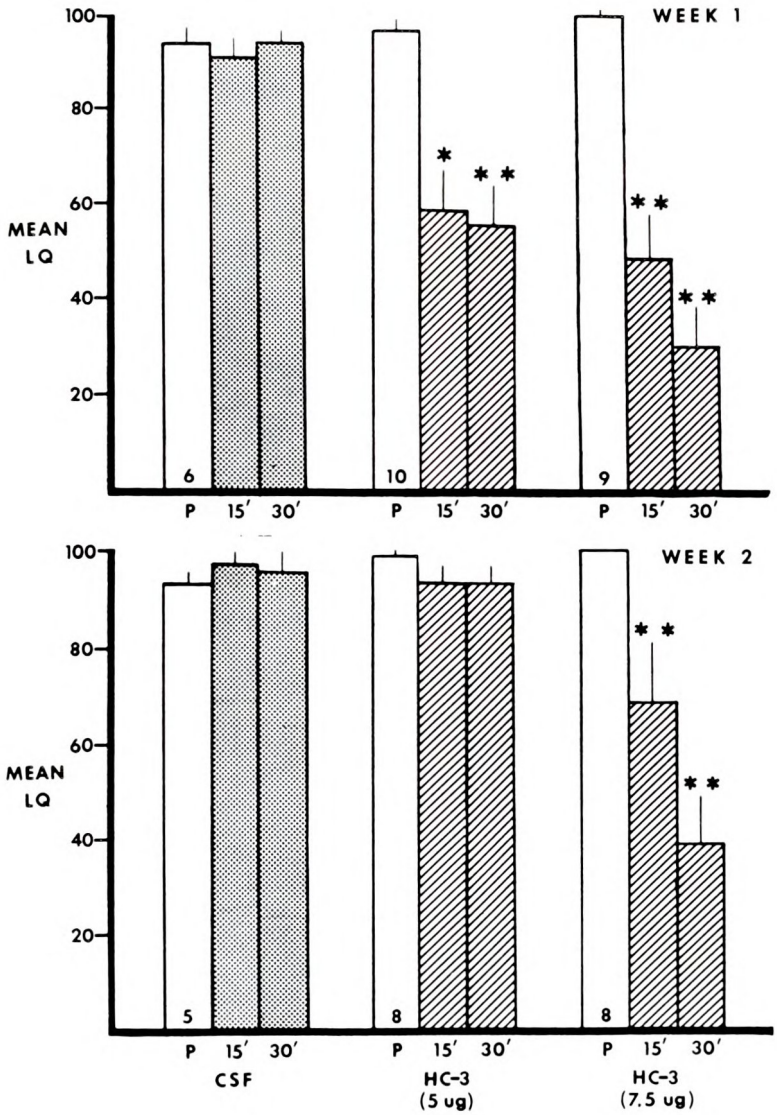


Figure 6. Mean lordosis quotients (LQ) recorded from female rats following bilateral infusion of hemicholinium-3 (HC-3, 5 or 7.5 ug/cannula) or artificial cerebrospinal fluid (CSF) vehicle into the bed nucleus of the stria terminalis. All females were injected with 2 ug estradiol benzoate 72, 48, and 24 hr before behavioral testing and 0.5 mg progesterone 4-5 hr before testing. Each female received the same intracerebral treatment over 2 wk of testing. Intracerebral treatment was administered immediately following the preinfusion test (P) and behavioral effects were tested 15 and 30 min after infusion. *$p < .05$, **$p < .01$ (change in LQ following HC-3 vs. change in LQ following CSF at corresponding test times, Mann-Whitney U).

of 5 ug HC-3/cannula failed to alter lordotic behavior during the second wk of treatment.

EXPERIMENT 3b

Significant reductions in lordosis were observed in Experiment 3a following a single infusion of HC-3 into the bed nucleus of the stria terminalis of receptive females. However, behavioral inhibition was evident only at the higher dose of HC-3 (7.5 ug/cannula) following a second treatment on the subsequent wk. Since steroids are known to reduce the effectiveness of various neurotropic agents (Selye, 1971a, b), the behavioral tolerance to repeated administration of HC-3 may be enhanced in animals treated with estrogen and progesterone. In order to facilitate the effects of HC-3 on lordosis and prevent the development of tolerance, lower doses of EB were utilized in Experiment 3b. In addition, the possibility of a stimulatory action of HC-3 was examined by testing females primed with a combination of EB and progesterone which normally activates only a low level of sexual receptivity.

METHOD

Ovariectomized female rats were bilaterally implanted in the POA according to the procedure outlined in the General Method section (See Figure 5). During the following 2 wk, females were injected intramuscularly with 0.175 ug, 0.25 ug, or 0.5 ug EB 72, 48, and 24 hr before behavioral testing. Progesterone (0.5 mg) was injected

intramuscularly 4-5 hr before testing. During the test session, each female was pretested for lordosis and afterward bilaterally infused with either HC-3 (5 ug/cannula) or CSF vehicle. The effects of intracerebral treatments on lordosis were tested 30 min after infusion. Each animal received the same priming dose of EB and intracerebral treatment during both wk of testing.

RESULTS

Preoptic infusion of HC-3 (5 ug/cannula) caused substantial reductions in lordotic behavior of females primed with EB and progesterone (Figure 7). The change in LQ following HC-3 infusion (pretest minus test) was compared to the change in LQ observed following CSF infusion at corresponding doses of EB (Mann-Whitney U test). During the first wk of treatment, significant reductions in LQ were seen following HC-3 infusion in females receiving 0.5 ug EB plus progesterone ($p < .01$) or 0.25 ug EB plus progesterone ($p < .01$). During the second wk of treatment, a significant reduction in LQ following HC-3 infusion was observed only in those females receiving 0.25 ug EB plus progesterone ($p < .01$). A second exposure to HC-3 failed to significantly reduce lordotic behavior in females receiving 0.5 ug EB plus progesterone. The tolerance to HC-3 displayed by females primed with this highest EB dose was similar, though not as pronounced, as that seen in Experiment 3a at a 2 ug priming dose of EB. Finally,

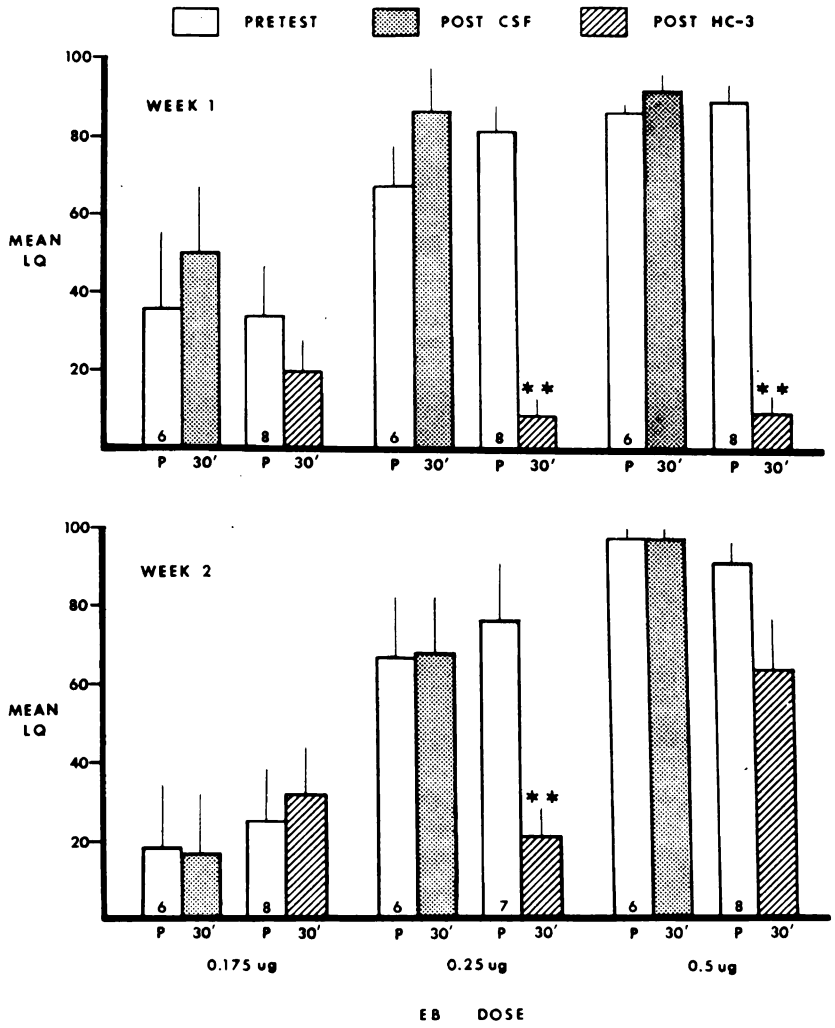


Figure 7. Mean lordosis quotients (LQ) recorded from female rats following bilateral infusion of hemicholinium-3 (HC-3, 5 ug/cannula) or artificial cerebrospinal fluid (CSF) vehicle into the medial preoptic area. Various doses of estradiol benzoate (EB) were injected 72, 48, and 24 hr before behavioral testing and 0.5 mg progesterone was injected 4-5 hr before testing. Each female received the same hormone-intracerebral treatment combination over 2 wk of testing. Intracerebral treatment was administered immediately following the preinfusion test (P) and behavioral effects were tested 30 min after infusion. $^{**}p < .01$ (change in LQ following HC-3 vs. change in LQ following CSF at corresponding EB doses, Mann-Whitney U).

no significant elevations in lordotic behavior occurred in HC-3-treated females receiving 0.175 ug EB plus progesterone.

EXPERIMENT 3c

Experiment 3c evaluated the specificity of the HC-3 effects reported in Experiments 3a and 3b. Choline, possibly the rate limiting factor in acetylcholine synthesis, is not produced centrally but rather is transported to the neuron in a lipid-bound form (Ansell and Spanner, 1968). The quaternary ammonium compound, HC-3, is postulated to competitively inhibit the transport of choline across neuronal membranes to the site of acetylcholine synthesis (MacIntosh et al., 1956; Gardiner, 1961; Guyenet et al., 1973). Accordingly, the depletion in brain acetylcholine content reported following intraventricular infusion of HC-3 (Freeman, Choi, and Jenden, 1975) can be prevented if choline chloride is infused along with HC-3 (Slater, 1968). In Experiment 3c, the ability of choline chloride to prevent the inhibition of lordosis by HC-3 was investigated. A dose of 120 ug/cannula of choline chloride was administered in order to reverse the effects of 5 ug/cannula of HC-3. Choline chloride doses 12-50 times those of HC-3 are required to prevent significant reduction in acetylcholine levels (Birks and MacIntosh, 1961; Slater 1968). A second control in Experiment 3c consisted of infusion of an active dose of HC-3 into cortical brain areas of females receiving estrogen and progesterone to

determine if HC-3 action was specific to particular brain sites. No alteration in lordotic behavior was expected from cortical infusion of HC-3 since this area does not concentrate ovarian hormones (Pfaff and Keiner, 1973).

METHOD

Ovariectomized female rats were bilaterally implanted in the POA according to the procedure outlined in the General Method section (See Figure 5). During the following wk, females were injected intramuscularly with 0.5 ug EB 72, 48, and 24 hr before behavioral testing. Progesterone was injected intramuscularly 4-5 hr before testing. During the test session, females were pretested for lordosis and afterward bilaterally infused with a CSF solution containing HC-3 (5 ug/cannula) or a CSF solution containing HC-3 (5 ug/cannula) plus choline chloride (120 ug/cannula). The effects of intracerebral treatments on lordosis were tested 30 and 60 min after infusion. A single test session was conducted.

Bilateral implants were also placed in the frontal cortex of ovariectomized females according to the procedure outlined in the General Method section (See Figure 5). During the following 2 wk, females were injected intramuscularly with 0.5 ug EB 72, 48, and 24 hr before behavioral testing. Progesterone was injected intramuscularly 4-5 hr before testing. During the test session, all females were pretested for lordosis and afterward bilaterally infused with HC-3 (5 ug/cannula). The effects of

intracerebral treatment on lordosis were tested 30 and 60 min after infusion.

RESULTS

When HC-3 (5 ug/cannula) was infused in combination with choline chloride (120 ug/cannula) the inhibitory effect of HC-3 on lordosis was arrested (Figure 8). The reductions in LQ observed at 30 and 60 min following HC-3 infusion were significantly greater than reductions seen following infusion of HC-3 plus choline chloride (at 30 min, $p < .01$; at 60 min, $p < .05$). The disruption of the inhibitory effect of HC-3 achieved by choline chloride began to wane by 60 min. There was no evidence of behavioral inhibition following infusion of HC-3 (5 ug/cannula) into the frontal cortex of females treated with EB and progesterone (Table 2).

SUMMARY

A cholinergic antagonist was infused into forebrain areas of ovariectomized female rats brought into sexual receptivity by administration of estrogen and progesterone. Bilateral infusion of HC-3 (5 or 7.5 ug/cannula), an acetylcholine synthesis inhibitor believed to interfere with choline uptake, decreased the incidence of lordotic behavior displayed by females. This inhibition was prevented by infusion of choline chloride (120 ug/cannula) along with HC-3. HC-3 did not inhibit lordosis when infused into the frontal cortex nor did HC-3 facilitate lordosis in unreceptive females following infusion into the POA.

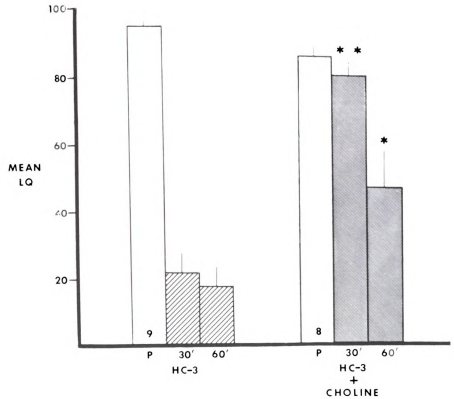


Figure 8. Mean lordosis quotients (LQ) recorded from female rats following bilateral infusion of hemicholinium-3 (HC-3, 5 ug/cannula) or HC-3 (5 ug/cannula) plus choline chloride (120 ug/cannula) into the medial preoptic area. All females were injected with 0.5 ug estradiol benzoate 72, 48, and 24 hr before behavioral testing and 0.5 mg progesterone 4-5 hr before testing. Intracerebral treatment was administered immediately following the preinfusion test (P) and behavioral effects were tested 30 and 60 min after infusion. *$p < .05$, **$p < .01$ (change in LQ following HC-3 vs. change in LQ following HC-3 plus choline, Mann-Whitney U).

TABLE 2

Mean Lordosis Quotients Recorded from Female Rats

Following Bilateral Infusion of Hemicholinium-3 into the Frontal Cortex

INTRACEREBRAL TREATMENT*	WEEK 1				WEEK 2			
	n	Pretest	30 min	60 min	n	Pretest	30 min	60 min
Hemicholinium-3 (5 ug/cannula)	8	88 ± 4	90 ± 3	89 ± 5	7	90 ± 4	93 ± 3	89 ± 3

*All females were injected with 0.5 ug estradiol benzoate 72, 48, and 24 hr before behavioral testing and 0.5 mg progesterone 4-5 hr before testing. The effect of intracerebral treatment on lordosis was tested 30 and 60 min after infusion.

EXPERIMENT 4. Reversal of Hemicholinium-3 Inhibition of Lordosis by Carbachol

In Experiments 1 and 2, stimulation of central cholinergic receptors by infusion of a receptor agonist, carbachol, activated lordosis in hormone-primed female rats. In Experiment 3, inhibition of acetylcholine synthesis by infusion of a synthesis blocker, HC-3, reduced the incidence of lordosis in hormone-primed female rats. In Experiment 4, central cholinergic receptors were stimulated by preoptic infusion of carbachol in an attempt to reverse the inhibitory effects of HC-3 on lordosis.

METHOD

Ovariectomized female rats were bilaterally implanted with cannulae in the POA according to the procedure outlined in the General Method section (See Figure 9). Behavioral tests were conducted 1 wk after stereotaxic surgery. Each female was injected at 72, 48, and 24 hr prior to testing with one of four EB doses: 0.13 ug, 0.17 ug, 0.25 ug, 0.5 ug. Progesterone (0.5 mg) was injected 4-5 hr before testing. During the test session, each female was pretested for lordosis and afterward bilaterally infused with a CSF solution containing HC-3 (1.25 ug/cannula) or a CSF solution containing HC-3 (1.25 ug/cannula) plus carbachol (0.5 ug/cannula). The effects of intracerebral treatments

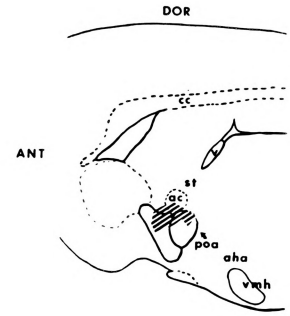


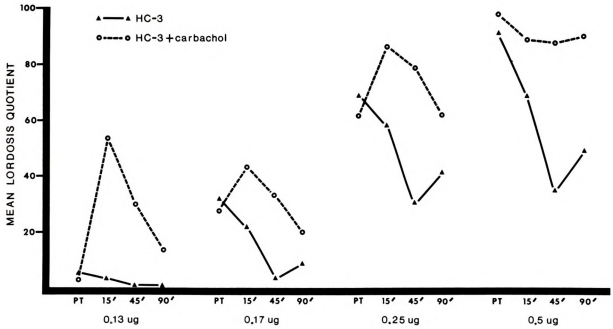
Figure 9. Distribution of cannula tips as verified histologically in female rats from Experiment 4. Only animals with implants in the areas indicated were included in data analysis. The plate represents a sagittal section taken 1.0 mm lateral to the midline. Adapted from Albe-Fessard et al. (1966). Abbreviations: ANT, anterior; DOR, dorsal; ac, anterior commissure; aha, anterior hypothalamic area; cc, corpus callosum; poa, preoptic area; st, stria terminalis; v, ventricle; vmh, ventromedial hypothalamus.

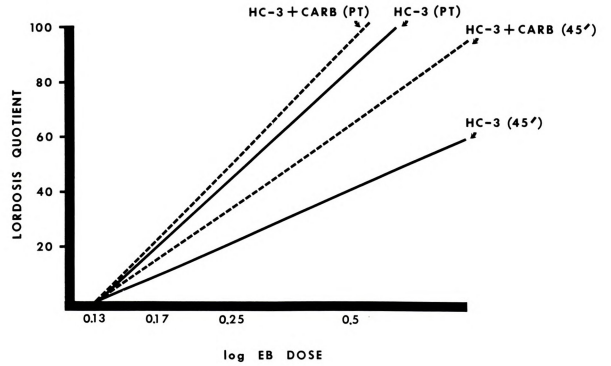
on lordosis were tested 15, 45, and 90 min after infusion. A single behavioral test session was conducted.

RESULTS

Carbachol (0.5 ug/cannula) blocked the inhibitory effect of HC-3 (1.25 ug/cannula) on lordosis in females primed with various doses of EB (0.13, 0.17, 0.25, 0.5 ug) in combination with progesterone (0.5 mg). Reduction in the incidence of lordosis was most pronounced 45 min after infusion of HC-3 at all doses of EB treatment (Figure 10, upper panel). In order to compare the effects of HC-3, and HC-3 plus carbachol, over all four doses of EB, linear regression lines of LQ on log transformed EB doses were generated. The lines illustrated in the lower panel of Figure 10 represent regression functions of pretest and 45 min test LQ recorded at the four doses of EB priming. Analysis of variance indicated that regression functions were statistically equivalent for pretests before HC-3, pretests before HC-3 plus carbachol, and tests 45 min after HC-3 plus carbachol. The regression line for tests 45 min after HC-3 was significantly different from the counterpart line for tests 45 min after HC-3 plus carbachol ($p < .05$). The regression line for tests 45 min after HC-3 was also significantly different from a regression line formed by pooling the regression functions of the three other lines ($p < .01$). In addition, at the 0.13 ug dose of EB, a substantial and transient increase in the incidence

Figure 10. Upper panel. Mean lordosis quotients recorded from female rats following bilateral infusion of hemicholinium-3 (HC-3, 1,25 ug/cannula) or HC-3 (1.25 ug/cannula) plus carbachol (0.5 ug/cannula) into the medial preoptic area. Various doses of estradiol benzoate (EB) were injected 72, 48, and 24 hr before behavioral testing and 0.5 mg progesterone was injected 4-5 hr before testing. Each female received only one behavioral test session. Intracerebral treatment was administered immediately following the preinfusion test (PT). The effect of intracerebral treatment on lordosis was tested 15, 45, and 90 min after infusion. For each treatment combination, n = 9-10. Lower panel. Linear regression lines of lordosis quotient on $\log_{10}$ transformed EB doses for pretests and 45 min tests.


HC-3
HC-3+carbachol
MEAN LORDOSIS QUOTIENT
100
80
60
40
20
PT 15′ 45′ 90′
0,13 ug
0,17 ug
0,25 ug
0,5 ug
EB PRIMING DOSE
(+0.5 mg PROGESTERONE)


HC-3+CARB (PT)
HC-3 (PT)
HC-3+CARB (45′)
HC-3 (45′)
LORDOSIS QUOTIENT
100
80
60
40
20
0.13
0.17
0.25
0.5
log EB DOSE

of lordosis was observed after infusion of HC-3 plus carbachol (Figure 10, upper panel), similar to that seen in Experiment 1 after infusion of carbachol alone.

Results indicate that the incidence of lordosis on pretests varied as a function of the dose of EB which was administered in conjunction with progesterone. This relationship between LQ and EB dose was maintained 45 min after infusion of HC-3 plus carbachol. In contrast, LQ was dependent on EB dose 45 min after infusion of HC-3 alone, but was reduced at all doses of EB priming.

Infusion of HC-3 consistently inhibited the display of soliciting behaviors, as well as lordosis. However, carbachol failed to maintain soliciting behaviors in females treated with HC-3 plus carbachol.

SUMMARY

In Experiment 4, the inhibition of lordosis by HC-3 (1.25 ug/cannula), an acetylcholine synthesis blocker, was prevented when carbachol (0.5 ug/cannula), a cholinergic receptor agonist, was infused along with HC-3.

EXPERIMENT 5. Alterations in Cholinergic Binding Sites within Specific Brain Areas Following Estrogen Treatment

The results of Experiments 1-4 indicate that pharmacological manipulations of the cholinergic system influence the sexual behavior displayed by female rats. In particular, stimulation of cholinergic receptors in certain brain regions increased the probability of lordosis, but only following pretreatment with low doses of estrogen (Experiments 1 and 2). This estrogen-dependent facilitation of lordosis by a cholinergic receptor agonist suggests that estrogen may alter specific cholinergic processes within the brain. Recent evidence has, in fact, confirmed an increase in cholinergic muscarinic binding in certain brain regions following estrogen treatment of female rats (Rainbow, DeGroff, Luine, and McEwen, 1980). Two days of systemic treatment with 10 ug EB increased in vitro muscarinic binding in whole hypothalami, as well as in more discrete preparations of VMH and anterior hypothalami.

In Experiment 5, muscarinic binding was analyzed in various brain areas from female rats which had been treated with several doses of estrogen (0.125, 1, or 10 ug EB for 3 days). Muscarinic agonists have been shown to facilitate lordosis following implantation or infusion into the POA or VMH of females primed with 0.13 ug EB (Experiments

1 and 2) or 1 ug EB (Clemens et al., 1980). It was suspected that these low priming doses of EB might increase the sensitivity of muscarinic receptors in the POA and VMH, thereby promoting the action of cholinergic receptor agonists. An increase in receptor sensitivity could result from alterations in receptor number or receptor affinity (Scatchard, 1949). Changes in muscarinic binding were assessed in vitro using ^{3}H quinuclidinyl benzilate (QNB), a high affinity muscarinic antagonist.

METHOD

Two wk after ovariectomy, female rats were injected intramuscularly with EB (0.125, 1, or 10 ug) or 0.1 cc sesame seed oil 72, 48, and 24 hr before sacrifice. Following decapitation, the brain was rapidly removed and various areas were dissected in ice-cold saline according to the method of Luine, Khylchevskaya, and McEwen (1974). Brain areas assayed included the POA, medial basal hypothalamus (MBH), and portions of the caudate nucleus and parietal cortex. Fresh tissues were homogenized in 1 ml of ice-cold 320 mM sucrose. Homogenates were centrifuged at 1000 g for 10 min at 4° C. Twenty-five ul aliquots of low-speed supernatant (5-30 ug protein) were incubated for 1 hr at 37° C in 207 ul of potassium phosphate buffer containing 320 mM sucrose, 10 mM potassium phosphate, 1 mM EDTA, and 0.5 mM ascorbate (pH 7.4), with 1 nM ^{3}H l-QNB (specific activity 40 Ci/mmole, New England Nuclear). The reaction was terminated by filtration through Whatman GF/B

filters under vacuum. Filters were transferred to glass scintillation vials and allowed to dry. Ten ml of Econofluor counting solution (New England Nuclear) was added and samples were counted 24 hr later at 45% efficiency. In order to determine the amount of nonspecific binding, parallel incubations were run in a potassium phosphate buffer containing 1 nM ^{3}H QNB and 1 uM atropine sulfate (Sigma Chemical Co.). Specific binding was defined as binding in the absence of atropine sulfate minus binding in the presence of atropine sulfate. Protein content of each sample was determined by the method of Bradford (1976) with bovine-serum albumin as standards. In this assay system, ^{3}H QNB binding increased linearly over a protein concentration range of 5-60 ug. Further, the cholinergic receptor agonist, carbachol, inhibited ^{3}H QNB binding (IC_{50} = 60 uM) as reported previously (Yamamura and Synder, 1974; Jones and Sumikawa, 1981). In order to differentiate changes in binding site number from changes in binding site affinity, isotherms were generated by incubating 25 ul aliquots of supernatant with several concentrations of ^{3}H QNB (0.025-1 nM).

RESULTS

The effects of EB treatment on ^{3}H QNB binding are presented in Figure 11 and Table 3. Systemic injection of EB for 3 days increased specific binding of ^{3}H QNB in the MBH of female rats at 1 ug ($p < .05$, vs. oil, two-tailed Mann-Whitney U-test following Kruskall-Wallis analysis of

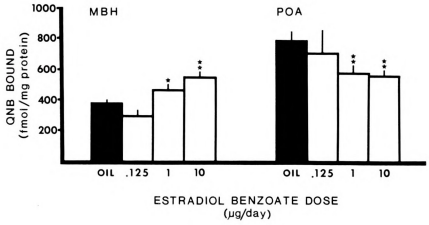


Figure 11. Specific binding of ^{3}H quinuclidinyl benzilate (QNB) in synaptosomal preparations of tissue from the medial basal hypothalamus (MBH; left) and medial preoptic area (POA; right) of female rats. Ovariectomized females were injected intramuscularly with estradiol benzoate (EB, 0.125, 1, or 10 ug) or 0.1 ml sesame seed oil 72, 48, and 24 hr before sacrifice (n = 5-11 per treatment). Specific binding was defined as total binding in the presence of 1 nM ^{3}H QNB minus nonspecific binding in the presence of 1 nM ^{3}H QNB and 1 uM atropine sulfate. ($\star p < .05$, $\star\star p < .01$; EB vs. oil, two-tailed Mann-Whitney U).

TABLE 3

Muscarinic Binding in the Parietal Cortex

and Caudate Putamen Following Estradiol Benzoate (EB) Treatment

AREA	OIL[1]	0.125 ug	1 ug	10 ug
Parietal Cortex	1546 ± 226[2]	1206 ± 122	1611 ± 109	1452 ± 121
Caudate Putamen	1778 ± 209	1605 ± 338	2009 ± 138	1823 ± 191

[1]EB or oil was administered 72, 48, and 24 hr before sacrifice (n = 6-11 per treatment).

[2]Values are expressed as fmol ^{3}H quinuclidinyl benzilate specifically bound per mg protein ± SEM. Details of the assay are described in the text. No significant changes in binding were detected at any dose of EB as indicated by Kruskall-Wallis analysis of variance.

variance) and 10 ug doses ($p < .01$). No significant alteration in ^3H QNB binding in the MBH was detected following treatment with 0.125 ug EB for 3 days. In contrast, administration of EB decreased specific binding of ^3H QNB binding in the POA at 1 and 10 ug doses ($p < .01$), but failed to alter binding at the 0.125 ug dose. EB treatment did not significantly affect ^3H QNB binding at any dose level in the parietal cortex or caudate putamen as indicated by Kruskall-Wallis analysis of variance (Table 3).

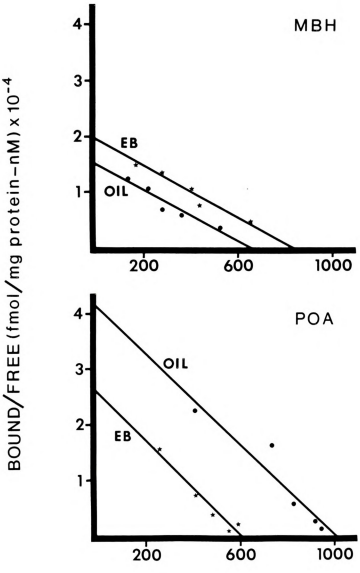
Analyses of the binding kinetics for MBH and POA tissue are presented in Figure 12. An estimate of binding site affinity is represented by the apparent dissociation constant or K_D (negative reciprocal of the slope). An estimate of binding site number per mg of protein is provided by the B_{max} (intercept of the x axis). EB (10 ug) increased the number of muscarinic binding sites per mg protein in the MBH, indicated by an increase in B_{max}, and decreased the number of muscarinic binding sites per mg protein in the POA, indicated by a decrease in B_{max}. EB did not alter binding site affinity (K_D) in either the MBH or POA.

SUMMARY

The results of Experiment 5 confirm the increase in muscarinic binding previously reported (Rainbow et al., 1980) in the MBH of female rats treated with 10 ug EB. A similar increase in binding was observed at 1 ug EB.

Figure 12. Scatchard plot of specific ^{3}H quinuclidinyl benzilate (QNB) binding in the medial basal hypothalamus (MBH; upper) and medial preoptic area (POA; lower) of female rats treated with estradiol benzoate (EB, 10 ug) or 0.1 ml sesame seed oil 72, 48, and 24 hr before sacrifice. Binding isotherms were generated by incubating synaptosomal preparations with several concentrations of ^{3}H QNB (0.025-1 nM). The free concentration of ^{3}H QNB present in the incubation volume was defined as total ^{3}H QNB added minus ^{3}H QNB specifically bound. K_D represents an estimate of binding site affinity. B_{max} represents an estimate of binding site number per mg of protein. Each point is the mean of 2-3 determinations.

	K_D (nM)		B_{max} (fmol/mg)	
	OIL	EB	OIL	EB
MBH	.044	.043	647	819
POA	.024	.023	1001	599


MBH
EB
OIL
4
3
2
1
200
600
1000
POA
OIL
EB
4
3
2
1
200
600
1000
BOUND/FREE (fmol/mg protein-nM) x 10⁻⁴
BOUND (fmol/mg protein)

However, muscarinic binding was not enhanced in the MBH following treatment with 0.125 ug EB, a priming dose found to be effective in behavioral Experiments 1 and 2. Furthermore, EB treatment (1 or 10 ug) actually reduced muscarinic binding in the POA. Scatchard analysis indicated that the alterations in muscarinic binding induced by estrogen were due to changes in the number of binding sites rather than affinity of binding sites.

DISCUSSION

Cholinergic activity in specific areas of the female rat brain may play a significant role in the regulation of sexual behavior. The potential of ovarian hormones to control biological functions by altering protein synthesis, and specifically neurotransmitter activity, forms the basis of this hypothesis. In the present series of experiments, female sexual behavior, indicated by the incidence of lordosis, was significantly increased in estrogen-primed female rats following bilateral infusion of a cholinergic receptor agonist, carbachol (0.5 ug/cannula), into the POA or VMH. The behavioral response was prompt and transient, occurring within 15 min after infusion and persisting for less than 90 min. At low levels of estrogen priming, intracerebral carbachol activated lordosis more effectively than systemic progesterone. Carbachol failed to facilitate lordosis following infusion into the MRF or frontal cortex, suggesting site-specificity. Furthermore, forebrain infusion of an agent which retards acetylcholine synthesis, HC-3 (1.25, 5, or 7.5 ug/cannula), reduced the incidence of lordosis in estrogen-progesterone-treated females. This action was specific to the cholinergic system since carbachol (0.5 ug/cannula) or choline chloride (120 ug/cannula) prevented the inhibition of lordosis by HC-3. Finally,

estrogen treatment (1 or 10 ug EB for 3 days) was found to increase cholinergic muscarinic binding in the MBH and decrease muscarinic binding in the POA. However, an expected increase in muscarinic binding within these areas was not observed at the estrogen-priming dose (0.125 ug) utilized in behavioral experiments.

These experiments confirm and extend recent findings which indicate that central cholinergic activity may influence hormonally-activated sexual behavior. An increase in lordotic behavior has been observed following systemic administration of nicotine to estrogen-primed females (Fuxe et al., 1977). This facilitative action of nicotine was prevented by prior treatment with a nicotinic receptor blocker but was unaltered by pretreatment with dopaminergic antagonists. More recently, muscarinic agonists in crystalline form were found to increase lordosis in estrogen-primed females when implanted into either the POA or the MRF (Clemens et al., 1980). The facilitation of lordosis by central stimulation of muscarinic receptors was blocked by pretreatment with atropine sulfate, a cholinergic muscarinic receptor blocker.

Earlier reports by Lindstrom described both facilitative and inhibitory roles of cholinergic stimulants in the mediation of lordosis. Cholinergic agonists pilocarpine, oxotremorine, and arecoline were initially found to reduce lordotic behavior in females treated with estrogen and progesterone (Lindstrom and Meyerson, 1967). A

serotonergic mechanism appeared to be implicated, however, since pretreatment with parachlorophenylalanine blocked the inhibitory effect of pilocarpine (Lindstrom, 1971). Pilocarpine and oxotremorine were later shown to increase lordotic behavior in estrogen-primed females 4 hr after systemic injection (Lindstrom, 1973). The facilitation was blocked by atropine sulfate, reinforcing a cholinergic interpretation. However, the enhancement of lordosis was attributed to a drug-induced release of adrenal progestins since adrenalectomy abolished the increase. The discrepancy between the effects observed by Lindstrom following systemic administration of cholinergic agents and data recorded in our laboratory may be related to the mode of drug delivery. Intracerebral administration of cholinergic agonists in specific brain areas has been found to activate a prompt lordotic response which persisted in the absence of the adrenal glands (Clemens et al., 1980). A facilitation of lordosis by manipulations of the cholinergic system appears to be predicated on the discrete chemical stimulation of certain areas of the rat brain.

In Experiment 1, bilateral infusion of carbachol into the POA facilitated lordosis in ovariectomized female rats under several estrogen and estrogen-progesterone regimens. Estrogen and progesterone were administered systemically; carbachol was administered intracerebrally. As shown in Figure 2, the various doses of estrogen (0.13, 0.17, and 0.25 ug EB) activated only low levels of lordosis even

when combined with progesterone (0.5 mg). However, when carbachol was infused into the POA (0.5 ug/cannula) of females receiving estrogen or estrogen and progesterone, a significant increase in the incidence of lordosis was observed. Carbachol facilitated lordosis at lower doses of estrogen priming than did progesterone. Furthermore, the combination of carbachol and progesterone did not increase the incidence of lordosis in estrogen-primed females beyond that achieved by carbachol alone. Although the possibility exists that the doses of carbachol and progesterone were not behaviorally comparable, even higher doses of progesterone (0.75 or 1 mg) fail to increase lordosis significantly at these low levels of estrogen priming (Clemens, Huang, and Caldwell, unpublished data). These doses of estrogen probably do not induce sufficient progestin receptors for progesterone to achieve a behavioral effect (Moguilewsky and Raynaud, 1977; Blaustein and Wade, 1978; MacLusky and McEwen, 1978). The disparity observed between the behavioral effects of carbachol and progesterone may indicate that carbachol and progesterone achieve their effects by separate mechanisms.

Lindstrom (1973) suggested that the ability of systemically administered cholinergic agonists, pilocarpine and oxotremorine, to facilitate lordosis was dependent upon a drug-induced release of adrenal steroids. In contrast, it has been reported that the lordotic response elicited by intracerebral treatment with muscarinic agonists persisted

in the absence of the adrenal glands (Clemens et al., 1980). Data from Experiment 1 confirm this result; systemic progesterone failed to facilitate lordosis at low levels of estrogen priming, whereas, intracerebral carbachol was effective at these same estrogen doses. It is doubtful that progesterone released from the adrenals would exert an action at low estrogen levels when an optimum dose of exogenous progesterone was ineffective.

The transient nature of the behavioral response to stimulation by carbachol also disputes the importance of adrenal steroid action. Maximal levels of lordosis were observed 15 min after intracerebral infusion of carbachol (Figure 2). The response began to wane at 45 min and had returned to control levels by 90 min. Lordosis activated by adrenal steroids would likely persist at high levels beyond 90 min after release. For example, the incidence of lordosis observed following ether-induced release of adrenal steroids in female rats has been found to increase linearly from the time of exposure to ether before peaking at 120-180 min (Franck, 1977). The prompt behavioral response to intracerebral treatment followed by a gradual decline to control levels suggests a discrete stimulation of central cholinergic receptors by carbachol.

In Experiment 2, the facilitation of sexual behavior by intracerebral infusion of carbachol was found to be a site-specific phenomenon. Specifically, bilateral infusion of carbachol (0.5 ug/cannula) into either the POA or the

VMH increased the incidence of lordosis in estrogen-primed female rats. However, carbachol failed to activate lordosis following infusion into the MRF or frontal cortex. The potential of carbachol to facilitate lordosis following infusion into a particular brain region may be related to the ability of that region to concentrate and respond to estrogen.

Intracerebral implants of estrogen have been shown to be effective in activating sexual behavior when located in the VMH (Barfield and Chen, 1977; Davis, McEwen, and Pfaff, 1979; Rubin and Barfield, 1980). It was suspected that carbachol might induce an even greater behavioral response following infusion into the VMH compared to the POA. However, the degree of facilitation of lordosis was similar following cholinergic stimulation of the VMH and POA at this dose of estrogen priming. Since other behavioral results suggest that the VMH is uniquely sensitive to estrogen, regional differences in the response of the POA and VMH to cholinergic stimulation may be evident at lower estrogen priming doses or lower carbachol doses.

Neither the MRF nor cortex concentrates estradiol as efficiently as the POA and VMH (Pfaff and Keiner, 1973). Furthermore, estrogen implants in the MRF facilitated lordosis only when combined with systemic progesterone (Ross et al., 1971; Yanase and Gorski, 1976). Since these areas appear to be less sensitive to estrogen it is not surprising that estrogen-dependent cholinergic effects were

not observed at the low dose of estrogen priming (0.13 ug EB for 3 days) found to be effective in the POA and VMH. Although microinfusion of carbachol in the MRF has proven to be ineffective, it has been reported previously that crystalline carbachol activated lordosis when implanted in the MRF of female rats primed with 1 ug EB for 3 days (Clemens et al., 1980). The effectiveness of crystalline carbachol in the MRF may be related to the high dose of carbachol delivered to brain tissue (approximately 10 ug/ cannula) and a potentially wider locus of stimulation.

A variety of evidence suggests that the POA and VMH exert different actions in mediating female sexual behavior. For example, although lesions of the VMH inhibited lordosis in estrogen-treated female rats (Mathews and Edwards, 1977), lesions of the POA increased the potential of estrogen-treated females to display lordosis (Powers and Valenstein, 1972). Furthermore, electrical stimulation of the VMH has been reported to facilitate lordosis (Pfaff and Sakuma, 1979) while stimulation of the POA inhibited lordosis (Malsbury and Pfaff, 1973). These results seem to suggest that the VMH exerts excitatory control over estrogen-induced lordosis and the POA exerts tonic inhibitory control. Appropriately, estrogen has been shown to increase the number of cells in the VMH displaying detectable spontaneous activity while decreasing their number in the POA (Bueno and Pfaff, 1976).

However, cholinergic stimulation of either the VMH or

POA under identical conditions facilitated lordosis in estrogen-treated female rats in Experiment 2. This finding may indicate that intracerebral stimulation with the cholinergic agonist carbachol activates an excitatory circuit in the VMH but suppresses an inhibitory circuit in the POA. Alternatively, both inhibitory and excitatory components of a neural circuit controlling lordosis could exist in the POA along with an excitatory component in the VMH. This latter suggestion is supported by the heterogeneous response of POA neurons observed during somatosensory stimulation of the perineum (Bueno and Pfaff, 1976).

The possibility that the facilitation of lordosis by chemical stimulation of the VMH or POA occurs after the cholinergic agent has diffused away from the site of infusion cannot be entirely dismissed at present. However, a behavioral response that results from the diffusion of carbachol across the brain substance which separates the POA and VMH seems unlikely. Available evidence indicates that, in general, drug diffusion is largely limited to a 1 mm radius around the infusion site when infusion volumes of less than 1 ul are employed (Rech, 1968; Myers, Tytell, Kawa, and Rudy, 1971).

In Experiment 3, a significant inhibition of sexual behavior was observed following intracerebral treatment with an agent known to disrupt cholinergic function. HC-3, a compound which retards acetylcholine synthesis (MacIntosh et al., 1956; Gardiner, 1961; Guyenet et al., 1973), reduced the

incidence of lordosis in estrogen-progesterone-treated females following bilateral infusion into the POA or stria terminalis. Similar reductions in the incidence of lordosis have been observed following systemic treatment with high doses (10 mg) of the cholinergic receptor antagonist, atropine sulfate (Singer, 1968). The ability of cholinergic antagonists such as HC-3 and atropine sulfate to inhibit lordosis in sexually receptive female rats indicates that cholinergic activity in specific areas of the brain may play an important role in the mediation of this hormone-dependent behavior.

The effect of HC-3 reported in Experiment 3 was both localized and specific. The inhibition of lordosis was observed only after HC-3 infusion into sites known to concentrate estrogen (Pfaff and Keiner, 1973). Furthermore, HC-3 failed to significantly reduce the incidence of lordosis when choline chloride was added to the infusate. The decline in acetylcholine levels following intraventricular infusion of HC-3 can be counteracted by simultaneous infusion of choline chloride (Slater, 1968). Evidence from <u>in vitro</u> preparations indicates that HC-3 inhibits the high affinity uptake of choline necessary for the intraneuronal synthesis of acetylcholine (MacIntosh et al., 1956; Gardiner, 1961; Guyenet et al., 1973). However, the mechanism by which HC-3 alters <u>in vivo</u> acetylcholine synthesis is not completely understood. Although intraventricular infusions of low doses of HC-3 (0.3-10 ug)

effectively deplete acetylcholine levels, concomitant inhibition of central ^{14}C-choline uptake has not been demonstrated (Robinson, 1970; Freeman et al., 1975). Choline chloride does prevent both depletion of acetylcholine levels (Slater, 1968) and high affinity choline uptake (Robinson, 1970) when infused with higher intraventricular doses of HC-3 (10-300 ug). A recent report (Freeman, Macri, Choi, and Jenden, 1979) suggests, however, that lower in vivo doses of HC-3 probably reduce endogenous acetylcholine levels by inhibiting high affinity uptake of choline. In Experiment 3c, the inhibitory effects of HC-3 (5 ug/cannula) on lordosis were counteracted by simultaneous administration of choline chloride (120 ug/cannula). This dose of HC-3 may have been effective in arresting choline uptake within a localized area around the cannula tip. Presumably the functional integrity of cholinergic processes within this region is vital to the full display of lordosis.

Behavioral inhibition was observed within 15 min after HC-3 infusion in Experiment 3a and within 30 min after HC-3 infusion in Experiments 3b and 3c. Similarly, whole brain acetylcholine levels have been reported to be significantly depleted within 10 min after intraventricular infusion of HC-3 (Slater, 1968), while lowest acetylcholine levels were detected after several hours (Freeman et al., 1975, 1979). In Experiment 3c, the inhibition of lordosis by HC-3 was completely prevented 30 min after infusion of HC-3 with choline chloride. However, by 60 min after infusion, the

behavioral inhibition by HC-3 was only partially arrested. This waning effect may have been the result of a rapid transport of excess choline from the brain (Ansell and Spanner, 1968).

A tolerance to the inhibitory effects of HC-3 was observed in some groups during the second wk of behavioral testing. This tolerance to HC-3 was prevented when the dose of HC-3 was increased (Experiment 3a) or when the priming dose of EB was decreased (Experiment 3b). Consequently, it seems likely that repeated steroid treatment induced a behavioral tolerance to HC-3 by the second wk of testing. This explanation seems most tenable since the occurrence of tolerance to HC-3 was clearly dependent upon the relative amount of EB administered and the period of exposure. Selye (1971 a, b) has demonstrated the protection afforded by steroids against toxic levels of neurotropic agents. Estrogen may heighten the activity of various enzyme systems which degrade and inactivate the neurotropic drug. Alternatively, repeated steroid exposure may have accelerated acetylcholine synthesis such that the lower dose of HC-3 (5 ug/cannula) was less effective on the second wk.

In Experiment 4, bilateral infusion of HC-3 (1.25 ug/cannula) into the POA also reduced the incidence of lordosis below pretest levels in females receiving various doses of EB (0.13, 0.17, 0.25, and 0.5 ug) and progesterone (0.5 mg). At each EB dose, the greatest degree of behavioral suppression was recorded 45 min after infusion of HC-3.

Endogenous acetylcholine levels are reported to be significantly depleted at 1 hr after intraventricular infusion of comparable quantities of HC-3 (Robinson, 1970). Lordosis was maintained at pretest levels when carbachol (0.5 ug/cannula) was infused along with HC-3 in females receiving 0.17, 0.25, and 0.5 ug EB with progesterone. Since both carbachol (Experiment 4) and choline chloride (Experiment 3c) were found to counteract the inhibitory effect of HC-3 on lordosis, a specific involvement of the cholinergic system is clearly indicated.

The ability of cholinergic manipulations to influence sexual behavior suggests that ovarian hormones alter cholinergic processes within the brain. Neuronal exposure to estrogen might promote acetylcholine synthesis, alter cholinergic receptor binding, or both. Recently, the activity of choline acetyltransferase was reported to be elevated significantly in the POA of ovariectomized female rats following EB treatment, suggesting increased acetylcholine synthesis (Luine, Khylchevskaya, and McEwen, 1975; Luine, Park, Tong, Reis, and McEwen, 1980). In Experiment 4, the contribution of acetylcholine synthesis was minimized by treating the POA with an agent, HC-3, which reduced intraneuronal synthesis of acetylcholine. Nevertheless, carbachol infusion maintained lordosis at pretest levels previously recorded for each dose of EB. Even in the absence of significant acetylcholine synthesis, carbachol facilitated lordosis in direct proportion to the priming

dose of EB. This observation may suggest that estrogen acts to enhance cholinergic processes other than synthesis. It is possible that estrogen increased the number or affinity of cholinergic receptors in the POA. Higher doses of EB (0.25 or 0.5 ug) would promote greater enhancement of cholinergic receptor sensitivity than lower doses of EB (0.13 or 0.17 ug), thereby accomodating greater behavioral effectiveness of a receptor mimetic such as carbachol. However, we cannot exclude the possibility that HC-3 reduced acetylcholine synthesis in varying degrees as a function of the dose of EB administered. Consequently, acetylcholine synthesis may be an important factor at certain EB doses, even in the presence of HC-3.

In order to determine directly whether estrogen alters the sensitivity of cholinergic muscarinic receptors in the brain, specific binding of a high-affinity radiolabelled muscarinic antagonist, ^{3}H QNB, was analyzed in discrete areas of the female rat brain in Experiment 5. Previous evidence indicated that high doses of estrogen (10 ug EB for 2 days) increased the number of muscarinic binding sites in whole hypothalamus, and specifically in the VMH and anterior hypothalamus (Rainbow et al., 1980). It was hypothesized that lower estrogen priming doses (0.125 or 1 ug EB for 3 days) used in behavioral experiments (Experiments 1 and 2, Clemens et al., 1980) would also increase muscarinic binding in the POA and MBH, and consequently facilitate the action of cholinergic receptor agonists.

However, in Experiment 5, the estrogen priming dose (0.125 ug EB) found to be effective in Experiments 1 and 2 failed to increase muscarinic binding in either the POA or MBH. Higher estrogen doses (1 or 10 ug EB) did increase binding in the MBH, confirming earlier findings (Rainbow et al., 1980). Scatchard analysis revealed that this alteration was the result of an increase in the number of muscarinic binding sites (B_{max}) rather than an increase in binding site affinity (K_D). Surprisingly, higher doses of estrogen (1 or 10 ug EB) decreased muscarinic binding in the POA, specifically by reducing the number of binding sites. There was no effect of estrogen treatment on muscarinic binding in two control areas, the parietal cortex and caudate putamen.

The failure to detect increases in muscarinic binding in the POA and MBH at the lowest estrogen dose (0.125 ug EB) may be due to the insensitivity of the assay system. Alternatively, alterations in the number of binding sites for a cholinergic agonist such as carbachol might not be evident when receptors are labelled with a cholinergic antagonist such as ^{3}H QNB. Evidence now indicates that muscarinic binding sites exist in three distinct forms with different affinities for agonists but similar affinities for antagonists (Birdsall, Burgen, Hiley, and Hulme, 1976). The distribution of the three agonist binding sites varies throughout the rat brain and high affinity sites have been suggested to represent degraded forms of the low affinity

site (Wamsley, Zarbin, Nigel, Birdsall, and Kuhar, 1980). Consequently, the effect of estrogen on the level of the biologically active low affinity receptor may be concealed in an assay system using a muscarinic antagonist such as ^{3}H QNB.

Although infusion of carbachol into the POA consistently facilitated lordosis in estrogen-primed females (0.125 ug EB), a decrease in muscarinic binding sites was observed in the POA following estrogen treatment at higher doses (1 or 10 ug EB). This result was quite unexpected and indicates that the cholinergic mechanism controlling lordosis may be complex. For example, evidence suggests that estrogen may not alter the same cholinergic parameters in the POA as in the MBH. In particular, the activity of CAT, the primary enzyme regulating acetylcholine synthesis, has been reported (Luine et al., 1975) to increase in the POA of female rats treated with estrogen (5 ug EB for 3 days). Since this hormonal regimen failed to alter CAT activity in the MBH, it appears that estrogen may modify cholinergic activity in different brain areas by different mechanisms. Thus, estrogen might promote cholinergic function in the POA by elevating CAT activity and consequently acetylcholine synthesis, and in the MBH by increasing the number of cholinergic binding sites. Together, present evidence suggests that cholinergic activity in the POA and VMH exerts an important influence of female sexual behavior and that estrogen promotes cholinergic function

in these areas, but by different mechanisms.

LIST OF REFERENCES

LIST OF REFERENCES

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

Albe-Fessard, D., Stutinsky, F., and Libouban, S. Atlas Stereotaxique du Diencephale du Rat Blanc. Paris: Centre National de la Recherche Scientifique, 1966.

Ansell, G. B., and Spanner, S. The metabolism of (Me-^{14}C) choline in the brain of the rat in vivo. Biochemical Journal, 1968, 110, 201-206.

Barfield, R. J., and Chen, J. J. Activation of estrous behavior in ovariectomized rats by intracerebral implants of estradiol benzoate. Endocrinology, 1977, 101, 1716-1725.

Birdsall, N. J. M., Burgen, A. S. V., Hiley, C. R., and Hulme, E. C. Binding of agonists and antagonists to muscarinic receptors. Journal of Supramolecular Structure, 1976, 4, 367-371.

Birks, R., and MacIntosh, F. C. Acetylcholine metabolism of a sympathetic ganglion. Canadian Journal of Biochemistry and Physiology, 1961, 39, 787-827.

Blaustein, J. D., and Wade, G. N. Progestin binding by brain and pituitary cell nuclei and female rat sexual behavior. Brain Research, 1978, 140, 360-367.

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

Bradford, M. M. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 1976, 72, 248-254.

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

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

Clemens, L. G. Neural plasticity and feminine sexual behavior in the rat. In Sex and Behavior, pp. 243-266. Edited by T. E. McGill, D. A. Dewsbury, and B. D. Sachs. New York: Plenum Press, 1978.

Clemens, L. G., and Christensen, L. W. Sexual behavior. In The Behaviour of Domestic Animals, pp. 108-145. Edited by E. S. E. Hafez. London: Bailliere Tindall, 1975.

Clemens, L. G., Humphrys, R. R., and Dohanich, G. P. Cholinergic brain mechanisms and the hormonal regulation of female sexual behavior in the rat. Pharmacology, Biochemistry, and Behavior, 1980, 13, 81-88.

Crowley, W. R., Feder, H. H., and Morin, L. P. Role of monoamines in sexual behavior of the female guinea pig. Pharmacology, Biochemistry, and Behavior, 1976, 4, 67-71.

Crowley, W. R., Nock, B. L., and Feder, H. H. Facilitation of lordosis behavior by clonidine in female guinea pigs. Pharmacology, Biochemistry, and Behavior, 1978, 8, 207-209.

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

Davis, P. G., McEwen, B. S., and Pfaff, D. W. Localized behavioral effects of tritiated estradiol implants in the ventromedial hypothalamus of female rats. Endocrinology, 1979, 104, 898-903.

Dohanich, G. P., and Clemens, L. G. Sexual behavior in female rats following intracerebral infusion of dopamine. Paper presented at the Eastern Conference on Reproductive Behavior, Rockefeller University, New York City, New York, 1980.

Eliasson, M. Action of repeated injections of LSD and apomorphine on the copulatory response of female rats. Pharmacology, Biochemistry, and Behavior, 1976, 5, 621-625.

Eliasson, M., and Meyerson, B. J. Comparison of the action of lysergic acid diethylamide and apomorphine on the copulatory response in the female rat. Psychopharmacology, 1976, 49, 301-306.

Eliasson, M., and Meyerson, B. J. The effects of lysergic acid diethylamide on copulatory behavior in the female rat. Neuropharmacology, 1977, 16, 37-44.

Eliasson, M., Michanek, A., and Meyerson, B. J. A differential inhibitory action of LSD and amphetamine on copulatory behavior in the female rat. Acta Pharmacologica et Toxicologica, 1972, 31, Supplementum 1, 22.

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

Everitt, B. J., and Fuxe, K. Dopamine and sexual behavior in female rats. Effects of dopamine receptor agonists and sulpiride. Neuroscience Letters, 1977, 4, 209-213.

Everitt, B. J., Fuxe, K., and 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., and Jonsson, G. Role of monoamines in the control by hormones of sexual receptivity in the female rat. Journal of Comparative and Physiological Psychology, 1975, 89, 556-572.

Foreman, M. M., and Moss, R. L. Role of hypothalamic serotonergic receptors in the control of lordosis behavior in the female rat. Hormones and Behavior, 1978a, 10, 97-106.

Foreman, M. M., and Moss, R. L. Role of hypothalamic alpha and beta adrenergic receptors in the control of lordotic behavior in the ovariectomized-estrogen primed rat. Pharmacology, Biochemistry, and Behavior, 1978b, 9, 235-241.

Foreman, M. M., and Moss, R. L. Role of hypothalamic dopaminergic receptors in the control of lordosis behavior in the female rat. Physiology and Behavior, 1979, 22, 283-289.

Franck, J. E. Short latency induction of lordosis by ether in estrogen-primed female rats. Physiology and Behavior, 1977, 19, 245-247.

Freeman, J. J., Choi, R. L., and Jenden, D. J. The effect of hemicholinium on behavior and on brain acetylcholine and choline in the rat. Psychopharmacology Communications, 1975, 1, 15-27.

Freeman, J. J., Macri, J. R., Choi, R. L., and Jenden, D. J. Studies on the behavioral and biochemical effects of hemicholinium in vivo. Journal of Pharmacology and Experimental Therapeutics, 1979, 210, 91-97.

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

Gardiner, J. E. The inhibition of acetylcholine synthesis in brain by a hemicholinium. Biochemical Journal, 1961, 81, 297-303.

Guyenet, P., Lefresne, P., Rossier, J., Beaujouan, J. C., and Glowinski, J. Inhibition by hemicholinium-3 of ^{14}C-acetylcholine synthesis and ^{3}H-choline high-affinity uptake in rat striatal synaptosomes. Molecular Pharmacology, 1973, 9, 630-639.

Hamburger-Bar, R., and Righter, H. Apomorphine: facilitation of sexual behavior in female rats. European Journal of Pharmacology, 1975, 32, 357-360.

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

Ho, K. W., Quadagno, D. M., Cooke, P. H., and Gorski, R. A. Intracranial implants of actinomycin D: Effects on sexual behavior and nucleolar ultrastructure in the rat. Neuroendocrinology, 1973, 13, 47-55.

Jones, S. W., and Sumikawa, K. Quinuclidinyl benzilate binding in house fly heads and rat brain. Journal of Neurochemistry, 1981, 36, 454-459.

Konig, J. F. R., and Klippel, R. A. The Rat Brain. Huntington, New York: Krieger, 1974.

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

Lindstrom L. H. The effect of pilocarpine and oxotremorine on oestrous behavior in female rats after treatment with monoamine depletors of 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., and Meyerson, B. J. The effect of pilocarpine, oxotremorine, and arecoline in combination with methylatropine or atropine 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.

Lisk, R. D., and Barfield, M. A. Progesterone facilitation of sexual receptivity in rats with neural implantation of estrogen. Neuroendocrinology, 1975, 19, 28-35.

Luine, V. N., Khylchevskaya, R. I., and McEwen, B. S. Oestrogen effects on brain and pituitary enzyme activities. Journal of Neurochemistry, 1974, 23, 925-934.

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

Luine, V., Park, D., Tong, J., Reis, D., and McEwen, B. Immunochemical demonstration of increased choline acetyltransferase concentration in rat preoptic area after estradiol administration. Brain Research, 1980, 191, 273-277.

McEwen, B. S., Davis, P. G., Parsons, B., and Pfaff, D. W. The brain as a target for steroid hormone action. In Annual Review of Neuroscience, Vol. 2, pp. 65-112. Edited by M. Cowan. Palo Alto, California: Annual Reviews Inc., 1979.

McGinnis, M. Y., Gordon, J. H., and Gorski, R. A. Influence of gamma-aminobutyric acid on lordosis behavior and dopamine activity in estrogen primed spayed female rats. Brain Research, 1980, 184, 179-191.

MacIntosh, F. C., Birks, R. I., and Sastry, P. B. Pharmacological inhibition of ACh synthesis. Nature, 1965, 178, 1181-1187.

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

Malsbury, C., and Pfaff, D. W. Suppression of sexual receptivity in the hormone-primed female hamster by electrical stimulation of the medial preoptic area. Proceedings of the Society for Neuroscience, 1973, abstract, 122.

Mathews, D., and Edwards, D. A. The ventromedial nucleus of the hypothalamus and hormonal arousal of sexual behaviors in the female rat. Hormones and Behavior, 1977, 8, 40-51.

Meyerson, B. J. Central nervous monoamines and hormone induced estrus behaviour in the spayed rat. Acta Physiologica Scandinavica, 1964, 63, Supplementum 241, 5-32.

Meyerson, B. J. The effect of imipramine and related antidepressive drugs on estrus behaviour in ovariectomised rats activated by progesterone, reserpine or tetrabenazine in combination with estrogen. Acta Physiologica Scandinavica, 1966, 67, 411-422.

Meyerson, B. J., Carrer, H., and Eliasson, M. 5-Hydroxytryptamine and sexual behavior in the female rat. Advances in Biochemical Psychopharmacology, 1974, 11, 229-242.

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

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

Moguilewsky, M., and Raynaud, J. P. Progestin binding sites in the rat hypothalamus, pituitary, and uterus. Steroids, 1977, 30, 99-109.

Myers, R. D., Tytell, M., Kawa, A., and Rudy, T. Microinjection of ^{3}H-acetylcholine, ^{14}C-serotonin, and ^{3}H-norepinephrine into the hypothalamus of the rat: Diffusion into tissue and ventricles. Physiology and Behavior, 1971, 7, 743-751.

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

O'Malley, B. W., and Means, A. R. Female steroid hormones and target cell nuclei. Science, 1974, 183, 610-620.

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

Pfaff, D. W., Nature of sex hormone effects on rat sex behavior: Specificity of effects and individual patterns of response. Journal of Comparative and Physiological Psychology, 1970, 73, 349-358.

Pfaff, D. W. Estrogens and Brain Function. New York: Springer-Verlag, 1980.

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

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

Powers, J. B., and Valenstein, E. S. Individual differences in sexual responsiveness to estrogen and progesterone in ovariectomized rats. Physiology and Behavior, 1972, 8, 673-676.

Quadagno, D. M., and Ho, G. K. W. The reversible inhibition of steroid-induced sexual behavior by intracranial cycloheximide. Hormones and Behavior, 1975, 6, 19-26.

Quadagno, D. M., Shryne, J., and Gorski, R. A. The inhibition of steroid induced sexual behavior by intrahypothalamic actinomycin D. Hormones and Behavior, 1971, 2, 1-10.

Rainbow, T. C., Davis, P. G., and McEwen, B. S. Anisomycin inhibits the activation of sexual behavior by estradiol and progesterone. Brain Research, 1980, 194, 548-555.

Rainbow, T. C., DeGroff, V., Luine, V. N., and McEwen, B. S. Estradiol 17beta increases the number of muscarinic receptors in hypothalamic nuclei. Brain Research, 1980, 198, 239-243.

Rech, R. The relevance of experiments involving injections of drugs into the brain. In Importance of Fundamental Principles in Drug Evaluation, pp. 325-360. Edited D. H. Tedeschi and R. E. Tedeschi. New York: Raven Press, 1968.

Robinson, C. A. Modification of acetylcholine and choline metabolism in the brain by hemicholinium-3. Ph.D. dissertation, Vanderbilt University, Nashville, Tennessee, 1970.

Rodgers, C. H., and Law, O. T. Effects of chemical stimulation of the "limbic system" on lordosis in female rats. Physiology and Behavior, 1968, 3, 241-246.

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

Rubin, B. S., and Barfield, R. J. Priming of estrous responsiveness by implants of 17 beta-estradiol in the ventromedial hypothalamic nucleus of female rats. Endocrinology, 1980, 106, 504-509.

Scatchard, G. The attractions of proteins for small molecules and ions. Annals of the New York Academy of Sciences, 1949, 51, 660-672.

Selye, H. Hormones and Resistance. New York: Springer-Verlag, 1971a.

Selye, H. Protection by estradiol against cocaine, coniine, ethylmorphine, LSD, and strychnine. Hormones and Behavior, 1971b, 2, 337-341.

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

Singer, J. J. The effects of atropine upon the female and male sexual behavior of female rats. Physiology and Behavior, 1968, 3, 377-378.

Slater, P. The effects of triethylcholine and hemicholinium-3 on the acetylcholine content of rat brain. International Journal of Neuropharmacology, 1968, 7,

Terkel, A. S., Shryne, J., and Gorski, R. A. Inhibition of estrogen facilitation of sexual behavior by the intracerebral infusion of actinomycin-D. Hormones and Behavior, 1973, 4, 377-386.

Uphouse, L. L., Wilson, J. B., and Schlesinger, K. Induction of estrus in mice: The possible role of adrenal progesterone. Hormones and Behavior, 1970, 1, 255-264.

Wamsley, J. K., Zarbin, M. A., Nigel, Birdsall, J. M., and Kuhar, M. J. Muscarinic cholinergic receptors: Autoradiographic localization of high and low affinity agonist binding sites. Brain Research, 1980, 200, 1-12.

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

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

Woo, S. L. C., and O'Malley, B. W. Hormone inducible messenger RNA. Life Sciences, 1975, 17, 1039-1048.

Yamamura, H. I., and Synder, S. H. Muscarinic cholinergic binding in rat brain. Proceedings of the National Academy of Science (Washington), 1974, 71, 1725-1729.

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

Young, W. C. The hormones and mating behavior. In Sex and Internal Secretions, Vol. 2, pp. 1173-1239. Edited by W. C. Young. Baltimore: Williams and Wilkins, 1961.

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