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Opiate Regulation of Lordosis: Neurochemical Evaluation of the Role of Opiate-Serotonergic Interactions

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OPIATE REGULATION OF LORDOSIS: NEUROCHEMICAL EVALUATION OF THE ROLE OF OPIATE-SEROTONERGIC INTERACTIONS

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SUMMARY

Opiates are important regulators of reproductive function in the rat, potently inhibiting gonadotropin secretion. However, the role of opiates in the control of lordosis, hormone-dependent female sexual behavior, is less clear. While opiate agonists inhibit lordosis, antagonists do not consistently facilitate behavior. The purpose of the present experiments was to further investigate opiate regulation of lordosis, and to examine the role of other neurotransmitter systems in the actions of opiates.

The opiate antagonist naltrexone was found to facilitate lordosis in estrogen-primed rats. Naltrexone-facilitated behavior was compared to the facilitation of behavior by progesterone. The medial preoptic area (POA) was found to be important in the actions of naltrexone.

The role of serotonergic systems in the opiate regulation of lordosis was examined. Lesions of hypothalamic serotonergic terminals blocked the inhibition of behavior by morphine. Naltrexone increased the rate of norepinephrine turnover, but decreased the rate of serotonin turnover in the POA. These changes were not seen in other areas of the brain. Both naltrexone and morphine increased the rate of serotonin synthesis in the POA. The dissociation between the rates of serotonin synthesis and turnover is discussed. Opiate regulation of serotonin

turnover was indirectly assessed by examining changes in serotonin receptors after treatment with opiates.

Opiates might be regulating serotonin release by actions at opiate receptors on serotonergic terminals in the POA. Levels of opiate receptors were measured after selective serotonergic lesions. Mu and delta opiate receptors in the POA were decreased after the lesion, suggesting that opiate receptors are located on serotonin terminals, and providing a neuroanatomical basis for opiate regulation of serotonin turnover.

The work presented here extends previous investigations of opiate regulation of lordosis. There is evidence that two previously described systems, serotonin and endogenous opiates, may be working in concert to regulate behavior.

LIST OF ABBREVIATIONS

5,7-DHT	5,7-Dihydroxytryptamine
5-HIAA	5-Hydroxyindoleacetic acid
5-HTP	5-Hydroxytryptophan
AH	Anterior hypothalamic nucleus
DAGO	[D-Ala^2,N-MePhe^4,-Gly-ol^5]Enkephalin
DMN	Dorsomedial nucleus of the hypothalamus
DPDPE	[D-Pen^2,D-Pen^5]Enkephalin
LH	Luteinizing hormone
LHRH	Luteinizing hormone releasing hormone
MCG	Midbrain central gray
NSD-1015	m-Hydroxybenzylhydrazine
p-PCA	p-Chlorophenylalanine
POA	Medial preoptic area
VMN	Pars lateralis of the ventromedial nucleus of the hypothalamus

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I. INTRODUCTION

A. LORDOSIS AND THE NEUROBIOLOGICAL ANALYSIS OF BEHAVIOR

The study of neurobiology is ultimately the study of behavior. The study of neurotransmitters, their related enzymes, and their regulation is the first step in understanding how changes in these systems alter what the animal does. Neurobiological research has concentrated on two classes of behaviors. The first can be broadly termed as learning, where the animal's behavior is modified by previous experience. The other class of behaviors are those that are innate to an animal, and are not readily modifiable, though the expression of these behaviors can show plasticity. These behaviors are the basic skills required for survival and reproduction, and are good models to study the neural regulation of behavior.

One innate behavior that has been well studied is lordosis, the response of female rodents to mounting by males. Lordosis is characterized by dorsiflexion of the spinal cord, extension of the front and rear legs, and movement of the tail to one side (Figure 1). Lordosis is an excellent behavior to study in order to understand the regulation of brain function and the neural control of behavior.

1. Lordosis is an important behavior in the reproductive cycle of rodents, and is required for

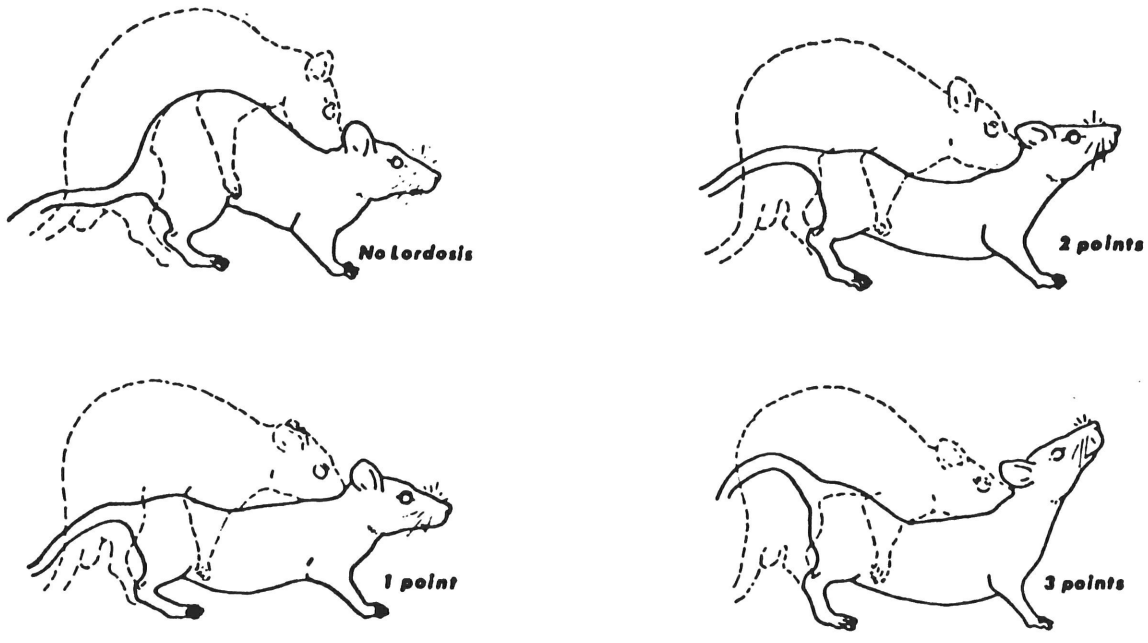


Figure 1. Rating system for scoring lordosis response by the female rat to mounting by a male (stippled outline). (From Hardy and DeBold, 1971).

fertilization. The dorsoflexion of the female's spine during lordosis allows for intromission by the male.

2. Lordosis behavior is easily quantifiable, with well established rating systems. The rating system of Hardy and Debold (1971), which will be described more completely in a later section, was used.

3. The areas of the nervous system important for the regulation of lordosis, and the connections between these areas, have been well studied. Most of this work has been done by Dr. Pfaff (1980) and collaborators. The basic circuitry can be divided into two parts.

The first part consists of the neurons and their connections in the periphery and the spinal cord that are directly involved in the reflex. These include sensory input from cutaneous receptors, afferents to the spinal cord, relays within the spinal cord and brain stem (Carrer, 1978), and motoneuron efferents to the muscles important for lordosis. This circuit describes a reflex, where the sensory input is provided by mounting of the male, and the response is the dorsiflexion by the female rat.

Higher brain areas are important for the expression of lordosis. Transections of the spinal cord drastically reduce lordosis responding (Hart, 1969; Kow, Montgomery and Pfaff, 1977), and remove the dependence of lordosis on hormones (Hart, 1969).

Primary among these areas are the preoptic area (Barfield and Chen, 1977; Pfaff and Sakuma, 1979a), the ventromedial hypothalamus (Barfield and Chen, 1977; Pfaff and Sakuma, 1979a, 1979b; Rainbow, McGinnis, Davis and McEwen, 1982, Etgen and Barfield, 1986), the midbrain central gray (Tennent, Smith and Davidson, 1982), and relay areas in the brainstem (Carrer, 1978). The role of these areas in the regulation of lordosis will be described later.

4. Lordosis is dependent on gonadal steroids. In intact cycling female rats, lordosis appears on the afternoon of proestrus and continues into estrus (Feder, 1981). Estrogen secretion by the ovaries increases during diestrus and proestrus (Butcher, Collins and Fugo, 1974). Levels of luteinizing hormone and progesterone, which are low over most of the estrous cycle, surge just before the appearance of sexual behavior. Estrogen and progesterone have been shown to be important for the induction of lordosis.

Removal of the ovaries, the site of estrogen and progesterone synthesis, completely abolishes lordosis responding. Sexual behavior can be reinstated by exogenous steroid hormone administration. Low doses of estradiol benzoate (3 to 5 ug) have minimal effects when sexual behavior is tested 24 to 42 hours after hormone

administration (Kow and Pfaff, 1975; Pfaff and Sakuma, 1979a). However, these low doses of estrogen are able to prime the female rats for the induction of behavior two to three hours after a subsequent injection of progesterone (Pfaff and Sakuma, 1979b). These two paradigms have been used to study the neurobiological basis of sexual behavior. The facilitation of lordosis by hormones, drugs, electrical stimulation or lesions can be studied in ovariectomized rats treated with low doses of estrogen, which alone do not facilitate behavior. The inhibition of sexual behavior can be studied in rats showing high levels of lordosis responding induced by both estrogen and progesterone. With these two models, it is possible to determine which hormones and neurochemicals are involved in mediating sexual behavior, where in the nervous system these chemicals exert their effects, and their mechanism of action.

B. HORMONAL REGULATION OF LORDOSIS

Steroid hormones regulate lordosis through actions in the brain (McEwen, Biegon, Davis, Krey, Luine, McGinnis, Paden, Parsons and Rainbow, 1982; Warembourg, 1985). The classical mechanism for steroid hormone action is by the hormone binding to intracellular receptors (Jensen and Jensen and DeSombre, 1973; Walters, 1985). The hormone-receptor complexes bind to DNA in the nucleus of the cell and regulate protein synthesis. Neurons that

accumulate gonadal steroids have been labeled by radioactive steroids, and identified by autoradiography (Pfaff and Keiner, 1973; Sheridan, Sar and Stumpf, 1974). Steroid accumulating cells are concentrated in the hypothalamus and limbic system (Pfaff and Keiner, 1973; Sheridan, Sar and Stumpf, 1974; Rainbow, Parsons, MacLusky and McEwen, 1982). Some of these areas have been shown to be important in the control of sexual behavior. The areas that are the most important for hormone-dependent facilitation of lordosis are the ventromedial nucleus of the hypothalamus (VMN, Barfield and Chen, 1977; Pfaff and Sakuma, 1979a, 1979b; Rainbow, McGinnis, Davis and McEwen, 1982, Etgen and Barfield, 1986), the medial preoptic area (POA, Powers and Valenstein, 1972; Barfield and Chen, 1977; Pfaff and Sakuma, 1979a) and the midbrain central gray (MCG, Pfeifle and Edwards, 1983; Tennent, Smith and Davidson, 1982; Akaishi and Sakuma, 1986). Lesions of the preoptic area facilitate or have minimal effects on sexual behavior (Law and Meagher, 1958; Powers and Valenstein, 1972), while electrical stimulation inhibits lordosis (Pfaff and Sakuma, 1979a). Destruction of the VMN blocks lordosis responding (Law and Meagher, 1958; Dorner, Docke and Hinz, 1969; Pfaff and Sakuma, 1979b), while stimulation of the VMN causes the appearance of sexual behavior (Pfaff and Sakuma, 1979a). The prime importance of the VMN in the regulation of hormone-dependent sexual behavior has been shown by studies in which sequential

placement of estrogen and then progesterone containing implants into the VMN were found to be sufficient to induce lordosis in ovariectomized rats (Rubin and Barfield, 1983). Thus, the VMN appears to be a facilitatory center for the regulation of sexual behavior, while the POA is inhibitory. Reciprocal connections exist between the POA and VMN (Simerly and Swanson, 1986; Conrad and Pfaff, 1975; Saper, Swanson, and Cowan, 1976). These connections appear to be important in the regulation of lordosis, since preoptic area lesions reduce the facilitation of behavior seen after estrogen implants into the VMN (Bast, Hunts, Renner, Morris and Quadagno, 1987).

Gonadal steroids are known to regulate neurotransmitter function in the brain, and this is thought to be one of the mechanisms by which steroids regulate female sexual behavior. Neurochemicals whose function is modulated by steroids, and which have been shown to be important regulators of lordosis include the monoaminergic neurotransmitters, serotonin (Meyerson, 1964a, 1964b, 1964c), norepinephrine (Davis and Kohl, 1977; Foreman and Moss, 1979; Fernandez-Guasti, Larsson and Beyer, 1985a, 1985b; Caldwell and Clemens, 1986) and dopamine (Foreman and Moss, 1979; Caggiula, Antelman, Chiodo and Lineberry, 1979; Caggiula, Herndon, Scanlon, Greenstone, Bradshaw and Sharp, 1979; Fernandez-Guasti, Ahlenius, Hjorth and Larsson, 1987), and peptides, such as LHRH (Moss and McCann, 1973; Pfaff,

1973), oxytocin (Gorzalka and Lester, 1987), prolactin (Harlan, Shivers and Pfaff, 1983) and beta-endorphin (Wiesner and Moss, 1984; Sirinathsinghji, 1984, 1986). The systems that were studied in these experiments are serotonin and the endogenous opiates.

C. SEROTONERGIC REGULATION OF LORDOSIS

Serotonin has been shown to be important for the hormone-dependent regulation of lordosis (Meyerson, 1964a, 1964b, 1964c; Everitt, Fuxe and Hokfelt, 1974, 1975; Fernandez-Guasti, Ahlenius, Hjorth and Larsson, 1987). Serotonergic neuron cell bodies are located in the midline raphe nuclei in the brainstem. Axons from these nuclei project rostrally to innervate the hypothalamus and preoptic area (Azmitia, 1978). In 1964, Meyerson investigated the role of monoaminergic neurotransmitter systems in the regulation of hormone- dependent female sexual behavior. Reserpine and tetrabenazine, which decreased the levels of monoamines in the brain, increased lordosis responding in estrogen-primed female rats (Meyerson, 1964c). Inhibitors of monoamine oxidase, which increase levels of monoamines by inhibiting their degradation, inhibit lordosis in estrogen and progesterone treated rats (Meyerson, 1964a, 1964b). Blocking the increase in monoamine levels after pargyline, a monoamine oxidase inhibitor, with H22/54 reduced the inhibition of lordosis by pargyline (Meyerson, 1964b).

Treatment with 5-hydroxytryptophan, the precursor of serotonin, potentiated the increase in monoamines after pargyline and the inhibition of lordosis (Meyerson, 1964a, 1964b). Meyerson (1964b) proposed the existence of monoaminergic pathways that inhibit lordosis, and suggested that serotonergic systems were particularly important for the monoaminergic inhibition of sexual behavior.

Serotonergic regulation of lordosis has been studied using pharmacological treatments that are more specific for serotonin. The precursor of serotonin, 5-hydroxytryptophan, increased serotonin levels and inhibited lordosis (Meyerson and Malmnas, 1978). Serotonin releasers or receptor agonists also decrease sexual behavior (Everitt, Fuxe and Hokfelt, 1975a; Everitt, Fuxe, Hokfelt and Jonsson, 1975b; Espino, Sano and Wade, 1975; Mendelson and Gorzalka, 1986a, Hlinak, 1987). Antagonists at postsynaptic serotonin receptors facilitate lordosis in estrogen-primed female rats (Zemlan, Ward, Crowley and Margules, 1973; Henrik and Gerall, 1976; Davis and Kohl, 1978; Rodriguez-Sierra and Davis, 1979). Possible sites for the serotonergic inhibition of lordosis have also been examined. Infusion of serotonergic antagonists into the preoptic area-anterior hypothalamus facilitate sexual behavior (Ward, Crowley and Zemlan, 1973; Zemlan, Ward, Crowley and Margules, 1973; Foreman and Moss, 1978a), while serotonin infusion inhibits lordosis (Foreman and Moss, 1978a). Serotonergic

antagonists also facilitate behavior when infused into the posterior hypothalamus (Ward, Crowley and Zemlan, 1973; Zemlan, Ward, Crowley and Margules, 1973) or the arcuate nucleus-ventromedial nucleus (Foreman and Moss, 1978a).

Lesions of serotonergic terminals in the hypothalamus induces lordosis in rats treated with low doses of estrogen, which by themselves do not facilitate behavior (Luine, Frankfurt, Rainbow, Biegon and Azmitia, 1983; Frankfurt, Renner, Azmitia and Luine, 1985). The facilitation of behavior by the lesion can be reversed by transplants of fetal raphe tissue, which contain serotonergic cell bodies (Luine, Renner, Frankfurt and Azmitia, 1984). The facilitation of behavior after the lesion could be due to the removal of an inhibitory serotonergic input, or the lesion could increase the sensitivity of hormone responsive neurons to steroid hormones. Work on catecholamines has suggested that monoaminergic activity may regulate responses to steroid hormones (Nock and Feder, 1981). Inhibition of norepinephrine synthesis or blockade of alpha-$_1$ noradrenergic receptors decreased the accumulation of nuclear estrogen receptors (Nock, Blaustein and Feder, 1981; Nock and Feder, 1984; Blaustein, 1985; Thornton, Nock, McEwen and Feder, 1986) and reduced the estrogen-dependent induction of progesterone receptors (Clark, Feder and Roy, 1985; Blaustein, Brown and Swearengen, 1986). The facilitation of behavior after serotonergic lesions does not

appear to be due to changes in sensitivity to estrogen, since the lesion did not alter the levels of nuclear estrogen receptors (O'Connor and Feder, 1983; Luine, Thornton, Frankfurt and MacLusky, 1987) or the induction of progesterone receptors by estrogen (Luine, Thornton, Frankfurt and MacLusky, 1987). Thus, serotonergic lesions in the hypothalamus facilitate lordosis. This facilitation of behavior is due to the removal of inhibitory serotonergic input, rather than due to an increase in the sensitivity of hypothalamic neurons to gonadal steroids.

The physiological role of serotonin dependent regulation of lordosis has been examined by measuring serotonin turnover in the hypothalamus after hormonal paradigms which result in the expression of lordosis behavior. Progesterone, which facilitates sexual behavior, also decreases serotonergic activity in the VMN (Johnson and Crowley, 1986; Renner, Krey and Luine, 1987). Changes in serotonergic activity in the hypothalamus are sufficient to alter the expression of lordosis, and are believed to be important for the gonadal steroid hormone dependent regulation of sexual behavior.

D. OPIATE REGULATION OF LORDOSIS

Exogenous opiates are also potent regulators of gonadotropin secretion (Bruni, van Vugt, Marshal and Meites, 1977; Cicero, Badger, Wilcox, Bell and Meyer, 1977;

Meites, Bruni, Van Vugt and Smith, 1979; Weisner, Koenig, Krulich and Moss, 1984; Bicknell, 1985) and female sexual behavior. Endogenous opioid peptides in the brain comprise three distinct opioid peptidergic systems, beta-endorphin, dynorphin and enkephalin. Each opioid peptide arises from a different precursor (Hughes, 1983), and they are located in distinct neuronal populations (Vincent, Hokfelt, Christensson and Terenius, 1982; McGinty and Bloom, 1983; Harlan, Shivers, Romano, Howells and Pfaff, 1987). Cell bodies for all three classes of opioid neurons are present in the hypothalamus (Hokfelt, Elde, Johansson, Terenius and Stein, 1977; Cuello, 1983; McGinty and Bloom, 1983; Morrell, McGinty and Pfaff, 1985; Yamano, Inagaki, Kito, Matsuzaki, Shinohara and Tohyama, 1986). Axons from these neurons project to the hypothalamus and extrahypothalamic areas (Dupont, Barden, Cusan, Merand, Labrie and Vaudry, 1980; Barden, Merand, Rouleau, Garon and Dupont, 1981; McGinty and Bloom, 1983; Watson, Hoffman and Wiegand, 1986; Yamano, Inagaki, Kito, Matsuzaki, Shinohara and Tohyama, 1986). Opioid peptides exert their effects through actions at opiate binding sites in the brain (Atweh and Kuhar, 1983). Different subtypes of opiate receptors exist (Martin, Eades, Thompson, Huppler and Gilbert, 1976), including mu, delta and kappa opiate receptors. Beta-endorphin binds to both mu and delta opiate receptors (Kosterlitz, Magnan and Paterson, 1982), while dynorphins bind to kappa receptors (Pfeiffer,

Pasi, Mehraein and Herz, 1981). The enkephalins bind to both mu and delta opiate receptors, but have a higher affinity for delta receptors (Paterson, Robson and Kosterlitz, 1983). Thus, endogenous opioid systems in the brain are complex (Lewis, Knachaturian and Watson, 1985), and a full understanding of the role of opioid peptides requires knowledge of each of the classes of opioid peptides and the use of drugs specific for the different subtypes of opiate receptors.

The role of opioid peptides in the regulation of sexual behavior has been examined using endogenous opioid peptides or drugs that specifically bind to opiate receptors. In rats, morphine (3 or 5 mg/kg), an opiate agonist, decreases lordosis quotients for one to two hours (Hetta, 1976), and the effect of morphine is blocked by the antagonist naltrexone. Intraventricular administration of beta-endorphin also inhibits receptive (lordosis quotients) and proceptive (ear-wiggling and hopping-and-darting) sexual behavior (Weisner and Moss, 1984; 1986a, 1986b). The inhibition of behavior is completely reversed by naloxone and the more specific u_1-opiate receptor antagonist naloxazone, and partially reversed by ICI 154,129, a delta-receptor antagonist (Weisner and Moss, 1986b). The effects of beta-endorphin do not appear to be due to a more generalized behavioral suppression, since beta-endorphin did not cause measurable catalepsy, alter blood pressure, or

affect the responsiveness to general somatosensory stimulation (Weisner and Moss, 1986a). Also, while beta-endorphin did decrease ambulation and grooming during an open field test, there was a lack of correlation with beta-endorphin inhibition of sexual behavior. The effects of beta-endorphin on open field behavior were completely blocked by ICI-154,129, which only partially blocks beta-endorphin's actions on sexual behavior (Weisner and Moss, 1986b). In golden hamsters, morphine decreases the frequency of lordosis and lateral displacement, the movement of the vaginal midline toward tactile stimulation of the perineal region (Ostrowski, Stapleton, Noble and Reid, 1979). Again, the inhibition of sexual behavior was blocked by naloxone.

However, in both rats and golden hamsters, the administration of opiate antagonists alone did not facilitate female sexual behavior in estrogen-primed animals (Ostrowski, Stapleton, Noble and Reid, 1979; Ostrowski, Noble and Reid, 1981; Pfaus and Gorzalka, 1987a). This suggested that while opiates are potent inhibitors of sexual behavior, endogenous opioid peptides are not tonically inhibiting lordosis.

E. PROPOSED STUDIES

In the work presented in this thesis, aspects of opiate regulation of sexual behavior were studied. The opiate

antagonist naltrexone was examined to determine if naltrexone is able to facilitate sexual behavior in estrogen-primed female rats. The behavioral actions of naltrexone were compared to the facilitation of behavior by progesterone. The inhibition of lordosis in estrogen and progesterone treated rats by the opiate agonist morphine was also investigated. The role of the medial preoptic area (POA) and the ventromedial nucleus of the hypothalamus (VMN), areas of the brain important for the hormone-dependent facilitation of lordosis, in the regulation of behavior by opiates was examined.

The role of other neurotransmitter systems, particularly serotonergic systems, in the opiate regulation of lordosis were also studied. Monoamine oxidase inhibitors, which increase levels of monoamines in the brain, block progesterone facilitated behavior. Pargyline inhibition of naltrexone facilitated behavior was also examined. Hypothalamic serotonergic lesions facilitate sexual behavior in estrogen primed female rats. The role of serotonergic systems in opiate regulation of lordosis was investigated by examining the ability of morphine to inhibit lordosis in rats with serotonergic lesions. If opiates regulate lordosis through actions on serotonergic terminals, the ability of morphine to inhibit behavior would be attenuated in rats with serotonergic lesions.

These results led to the hypothesis that opiates regulate serotonergic activity in areas of the brain important for the regulation of lordosis, and that this is one of the mechanisms by which opiates modify behavior. Opiate modulation of serotonin activity was assessed directly using biochemical turnover methods and indirectly by examining changes in serotonin receptors after treatment with opiates.

Lastly, a possible neuroanatomical basis for opiate regulation of serotonin turnover was examined. Opiates might be altering serotonin release by actions at opiate receptors on serotonin terminals. Levels of opiate receptors were measured after selective serotonergic lesions. Changes in opiate receptors would provide further evidence for opiate-serotonergic interactions in the brain, and would suggest that a subpopulation of opiate receptors are located on serotonergic terminals.

II. OPIATE REGULATION OF SEXUAL BEHAVIOR

A. NALTREXONE FACILITATION OF LORDOSIS

Introduction

Most previous studies have used naloxone as the opiate antagonist, and have tested behavior shortly after administration of naloxone (Ostrowski, Stapleton, Noble and Reid, 1979; Ostrowski, Noble and Reid, 1981; Pfaus and Gorzalka, 1987). While naloxone is highly selective for opiate receptors, it has a short half-life and duration of action in the body (Bonnet, Alpert, and Klinerock, 1978). The facilitation of lordosis in estrogen-primed female rats by subcutaneous injection of progesterone requires two to three hours (Glaser, Rubin and Barfield, 1983; Allen, Renner and Luine, 1985), though intravenous administration of progesterone has been reported to facilitate behavior within thirty minutes (Meyerson, 1972; Kubli-Garfias and Whalen, 1977; Glaser, Rubin and Barfield, 1983). The facilitation of lordosis behavior by opiate antagonists might require long-term blockade of opiate receptors. Naloxone may be unable to facilitate lordosis because it does not block opiate receptors for a sufficient period of time.

Opiate antagonists other than naloxone have been developed. One, naltrexone, has a longer half life after injection (Bonner, Alpert and Klinerock, 1978). Naltrexone can block opiate receptors for several hours. This long-term blockade of opiate receptors may be required for

the facilitation of lordosis. Thus, the effects of naltrexone on lordosis behavior were examined in estrogen-primed female rats, and possible mechanisms for its actions were examined.

Methods

Ovariectomized female rats were injected subcutaneously with 5 ug of estradiol benzoate in 0.1 ml of sesame oil. Forty-four hours later, rats were injected with saline, naltrexone hydrochloride (1, 3 or 5 mg/kg) in saline, or progesterone (0.5 mg in propylene glycol). Naltrexone hydrochloride was the kind gift of Dr. Rao Rapaka of the National Institute of Drug Abuse and Dr. Elliot Hahn. Sexual behavior was tested at various times after treatment. Each rat was tested only once each test day to minimize any effects of repeated testing.

Results

In estrogen-treated female rats, progesterone produced a significant increase in lordosis quotients within three hours, which was maintained for 5.5 hours after injection (Table 1). Naltrexone hydrochloride (3 mg/kg) given to estrogen-primed rats, produced a facilitation of sexual behavior after three hours, which was maintained for one hour. Overall, lordosis quotients were lower in naltrexone-treated rats than in animals treated with progesterone. A lower dose of naltrexone (1 mg/kg) did not facilitate behavior. Sexual behavior was facilitated in

TABLE 1
Effects of Naltrexone and Progesterone on Lordosis Quotients

Pharmacological treatment	Hours after treatment				
	0.5	2	3	4	5.5
Saline	20 ± 14 (4)	14 ± 6 (7)	10 ± 10 (5)	14 ± 7 (7)	25 ± 10 (4)
NTX (1 mg/kg)		8 ± 5 (4)	10 ± 6 (5)	3 ± 3 (4)	
NTX (3 mg/kg)	26 ± 16 (7)	23 ± 14 (4)	61 ± 9* (16)	45 ± 9* (13)	20 ± 6 (13)
NTX (5 mg/kg)		36 ± 14 (7)	25 ± 14 (8)	8 ± 4 (3)	
PROG (500 μg)		36 ± 13 (5)	93 ± 5* (9)	88 ± 10* (5)	78 ± 11* (8)

Note. Rats were injected with estradiol benzoate (5 μg), and 44 hr later were injected with saline, naltrexone hydrochloride (NTX), or progesterone (PROG). Sexual behavior was tested at various times after the second injection. Values for the lordosis quotient are the means ± SEM. The number of animals in each group is shown within the parentheses. Data were initially analyzed across treatments with the Kruskall–Wallis one-way ANOVA. If this test was significant, differences between individual groups were tested by the Mann–Whitney *U*-test.

* $P < 0.05$ compared to saline. Lordosis quotients for the progesterone-treated rats were significantly higher ($p < 0.05$) than for all other groups at 3, 4, and 5.5 hr.

three out of eight rats three hours after injection with 5 mg/kg naltrexone, although there was no significant difference between this group and the saline controls (data not shown). In rats without estrogen pretreatment, naltrexone (3 mg/kg after three hours) had no effect on sexual behavior (mean LQ $\pm$ SEM; 3 $\pm$ 3).

Discussion

Thus, naltrexone facilitates sexual behavior in estrogen primed female rats (Allen, Renner and Luine, 1985). This suggests that endogenous opiate peptides tonically inhibit lordosis. The blockade of opiate receptors by naltrexone removes this inhibition, and allows for the expression of behavior.

While many investigators have reported the inhibition of sexual behavior by opiate agonists which is reversed by antagonists (Ostrowski, Stapleton, Noble and Reid, 1979; Weisner and Moss, 1986b; Pfaus and Gorzalka, 1987a), most have also reported the failure of opiate antagonists alone to facilitate behavior in estrogen primed female rats (Ostrowski, Stapleton, Noble and Reid, 1979; Ostrowski, Noble and Reid, 1981; Pfaus and Gorzalka, 1987a). This inability of other investigators to detect the facilitation of lordosis by opiate antagonists may be due to the doses and route of administration of the drugs that were used, and the time when behavior was examined. In the results presented here, facilitation of behavior was consistently

observed only with 3 mg/kg naltrexone hydrochloride from three to four hours after injection. Hetta (1976) reported that 5 mg/kg naltrexone neither facilitated nor inhibited the lordotic response in estrogen-progesterone primed rats, but control levels of responding were 80%. With this high levels of responding, it may not have been possible to further facilitate behavior. Weisner and Moss (1984) reported that naloxone (2 or 40 mg/kg) did not facilitate lordosis up to 135 minutes after subcutaneous injection. Intracerebroventricular infusion of naloxone has been shown to be ineffective at inducing lordosis (Wiesner and Moss, 1984; Lindblom, Forsberg and Sodersten, 1986).

The bell-shaped dose response curve and the latency before behavior were two unexpected aspects of the naltrexone facilitation of behavior. Only one dose, 3 mg/kg naltrexone hydrochloride, effectively stimulated behavior. Neither a higher nor a lower dose reliably facilitated behavior. A similar U-shaped dose response relationship was reported for the effects of naltrexone on male sexual behavior (Myers and Baum, 1979). One explanation was that in addition to blockade of opiate receptors, we may also be detecting agonist properties of naltrexone (Ronai, Foldes, Hahn and Fishman, 1977).

The existence of different subtypes of opiate receptors may also be an important factor in the steep dose response curve. While naltrexone is selective for opiate receptors,

it shows little selectivity among the different subtypes of opiate receptors, particularly at the doses that were used. Different opiate receptors may have different actions on the expression of lordosis. Agonists at one subtype of receptor may inhibit lordosis, while agonists at another type of receptor may facilitate behavior. The facilitation of behavior observed after naltrexone may be the result of both facilitatory and inhibitory actions of naltrexone. The mediation of sexual behavior by different opiate receptor subtypes will be discussed more fully in the discussion.

B. FACILITATION OF LORDOSIS BY NALTREXONE IN ADRENALECTOMIZED RATS

Introduction

Unlike other actions of naltrexone which occur rapidly, the facilitation of lordosis after systemic injection of naltrexone has a latency of three hours. This is similar to the behavioral latency of subcutaneous injection of progesterone. However, the usual source of progesterone, the ovaries, have been removed. Another source of progesterone is the adrenal glands. Several drugs that facilitate lordosis, such as reserpine, have been found to act in part by causing the release of progesterone from the adrenal glands in sufficient quantities to facilitate behavior (Meyerson, 1964c; Paris, Resko and Gal, 1971). The opiate antagonist naloxone stimulates the release of ACTH

from the pituitary and increases plasma corticosterone levels (Eisenberg, 1980; Jezova, Vigas and Jurcovicova, 1982; Siegel, Chowers, Conforti, Feldman and Weidenfeld, 1982; Nilcolarakis, Pfeiffer, Stalla and Herz, 1987). Naltrexone may be indirectly facilitating sexual behavior through actions on the adrenal glands. In order to verify that the effects of naltrexone on sexual behavior were due to direct actions on the brain, and not indirectly through the adrenal glands, the facilitation of behavior by naltrexone was examined in ovariectomized and adrenalectomized rats.

Methods

Female rats were ovariectomized, or ovariectomized and adrenalectomized. Rats were injected with estradiol benzoate (5 ug), and forty-four hours later were injected with naltrexone hydrochloride (3 mg/kg). Sexual behavior was tested three hours later.

Results

Naltrexone facilitated sexual behavior in both ovariectomized and in ovariectomized and adrenalectomized rats (Figure 2). Lordosis quotients were higher in the ovariectomized-adrenalectomized rats, though this difference was not significant.

Discussion

Thus it appears that the facilitation of behavior by naltrexone is not due to the release of adrenal

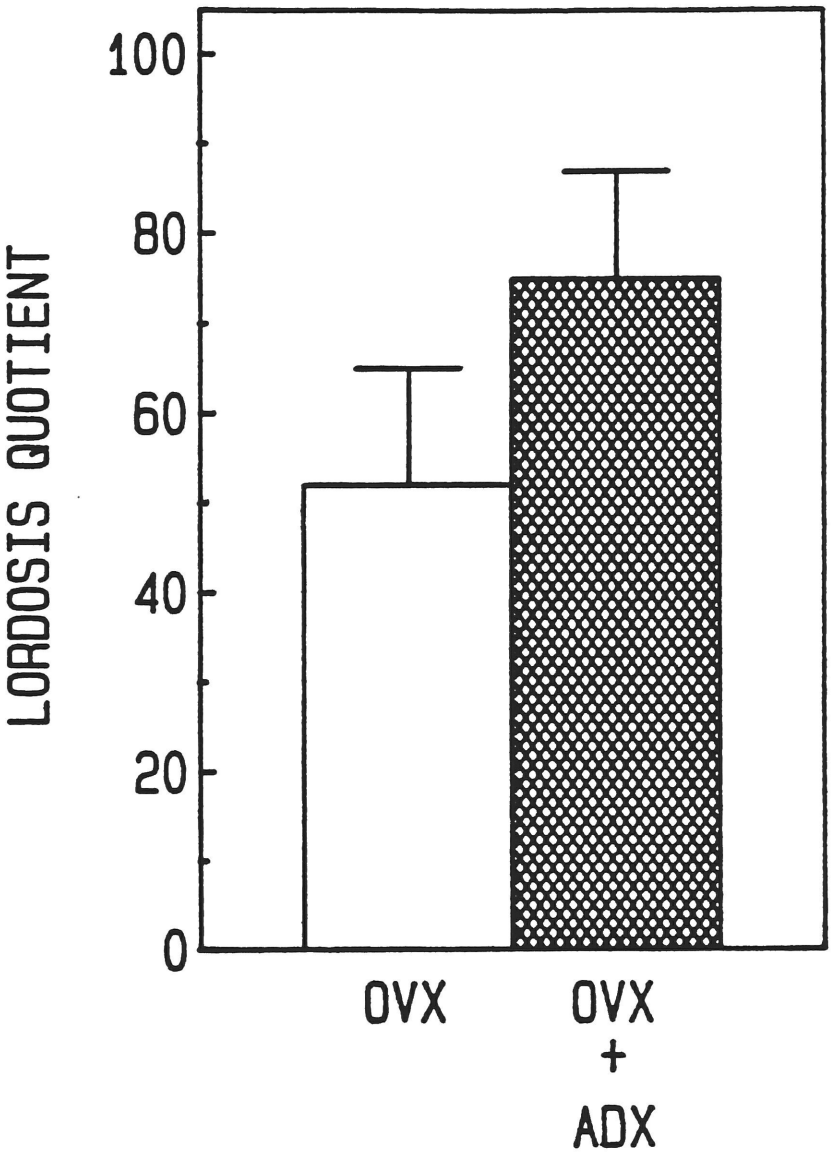


Figure 2. Facilitation of lordosis by naltrexone in adrenalectomized rats.

Ovariectomized rats were adrenalectomized (crosshatched bars), or sham-adrenalectomized (open bars). Seven days after surgery, rats were injected with estradiol benzoate (5 ug), and 44 hours later with naltrexone (3mg/kg). Sexual behavior was tested 3 hours later. Results are mean $\pm$ S.E.M., n = 6 for each group.

progesterone, but instead is due to direct actions of naltrexone on the nervous system.

C. ROLE OF PROTEIN SYNTHESIS IN THE ACTIONS OF NALTREXONE

Introduction

Facilitation of sexual behavior by estrogen and progesterone can be blocked by protein synthesis inhibitors such as anisomycin, which blocks protein chain elongation (Rainbow, Davis and McEwen, 1980). Steroid hormones enter cells and bind to intracellular receptors (Walters, 1985). The hormone receptor complexes bind to DNA in the nucleus of the cell, and regulate protein synthesis, which is considered to be their major mechanism for regulating behavior (Rainbow, Davis and McEwen, 1980; Rainbow, McGinnis, Davis and McEwen, 1982; Parsons, Rainbow, Pfaff and McEwen, 1982; Renner, Smits, Quadagno and Hough, 1984; Meisel and Pfaff, 1985). A drug, such as naltrexone, which acts directly at post-synaptic intramembrane receptors would not be expected to involve protein synthesis in its mechanism of action.

Method

To determine if the action of naltrexone on sexual behavior requires protein synthesis, the ability of naltrexone to facilitate lordosis was tested after the inhibition of protein synthesis. Forty-four hours after estradiol benzoate (5 ug), rats were injected with

anisomycin (80 mg/kg s.c. as a 2% solution in acidified saline) or vehicle. This dose of anisomycin inhibits protein synthesis in the central nervous system by at least eighty percent and antagonizes progesterone facilitated lordosis (Parsons, Rainbow, Pfaff and McEwen, 1982). Naltrexone (3 mg/kg) or progesterone (500 ug) were injected fifteen minutes later, and sexual behavior was tested after three hours.

Results

Both naltrexone and progesterone facilitated sexual behavior in estrogen primed female rats, though lordosis quotients were higher after progesterone (Figure 3). Anisomycin reduced lordosis scores after progesterone by more than fifty percent, but did not inhibit naltrexone-facilitated behavior.

Discussion

The inhibition of protein synthesis by anisomycin antagonizes the facilitation of behavior by progesterone in estrogen treated female rats. Naltrexone-facilitated sexual behavior does not appear to require protein synthesis, since anisomycin pretreatment did not decrease lordosis behavior.

D. INHIBITION OF LORDOSIS BY MORPHINE

Introduction

The opiate antagonist naltrexone facilitates female sexual behavior in the estrogen-primed rat. Previous work

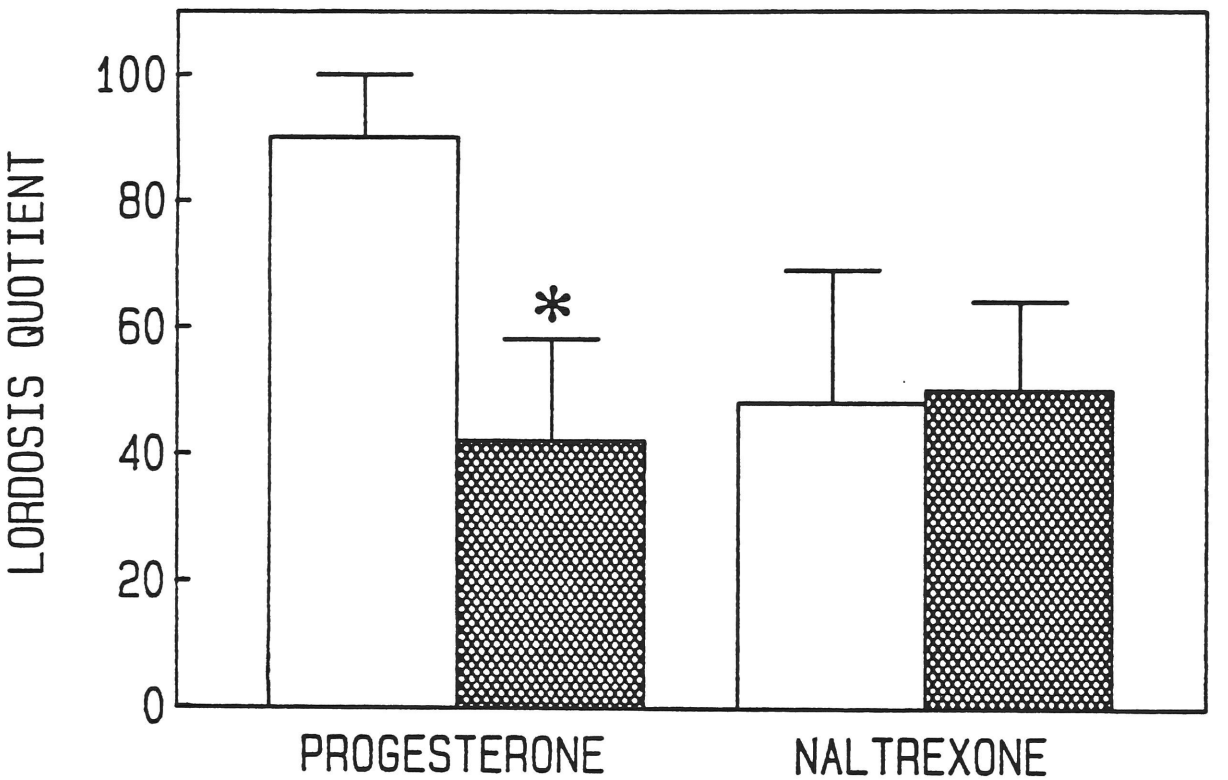


Figure 3. Effect of protein synthesis inhibition on the facilitation of lordosis by progesterone and naltrexone.

Ovariectomized female rats were injected with 5 ug estradiol benzoate. Forty-four hours later, rats were injected with progesterone (500 ug) or naltrexone hydrochloride (3 mg/kg), and sexual behavior was tested three hours later. Anisomycin (80 mg/kg, crosshatched bars) or vehicle (open bars) was injected 15 minutes prior to naltrexone or progesterone. Values are the mean $\pm$ s.e.m. for 4 to 6 rats.

* $p < 0.05$ compared to vehicle treatment using Mann-Whitney U-test.

by other investigators has shown that beta-endorphin and morphine, opiate agonists, inhibit sexual behavior in estrogen and progesterone treated female rats (Ostrowski, Stapleton, Noble and Reid, 1979, Weisner and Moss, 1986a, 1986b; Pfaus and Gorzalka, 1987a). In order to establish a baseline for further studies on the effects of opiates on sexual behavior, the effect of morphine on lordosis was examined.

Methods

Ovariectomized female rats were injected with estradiol benzoate (5 ug) and progesterone (500 ug 44 hours later). Rats were tested for lordosis behavior four hours after progesterone. After behavior, rats were injected subcutaneously with morphine sulfate or saline vehicle. Morphine sulfate wa the kind gift of Dr. Jules Hirsh of the Rockefeller University. Sexual behavior was tested again ten to twenty minutes later.

Results

Morphine sulfate inhibited sexual behavior in a dose dependent manner (Figure 4). Doses of morphine less than 1.6 mg/kg were ineffective. The highest dose, 8 mg/kg, completely inhibited sexual behavior. However with this dose, locomotor activity was greatly suppressed, and the inhibition of behavior was not specific to sexual behavior. At lower doses, sexual behavior was inhibited without gross effects on locomotor activity.

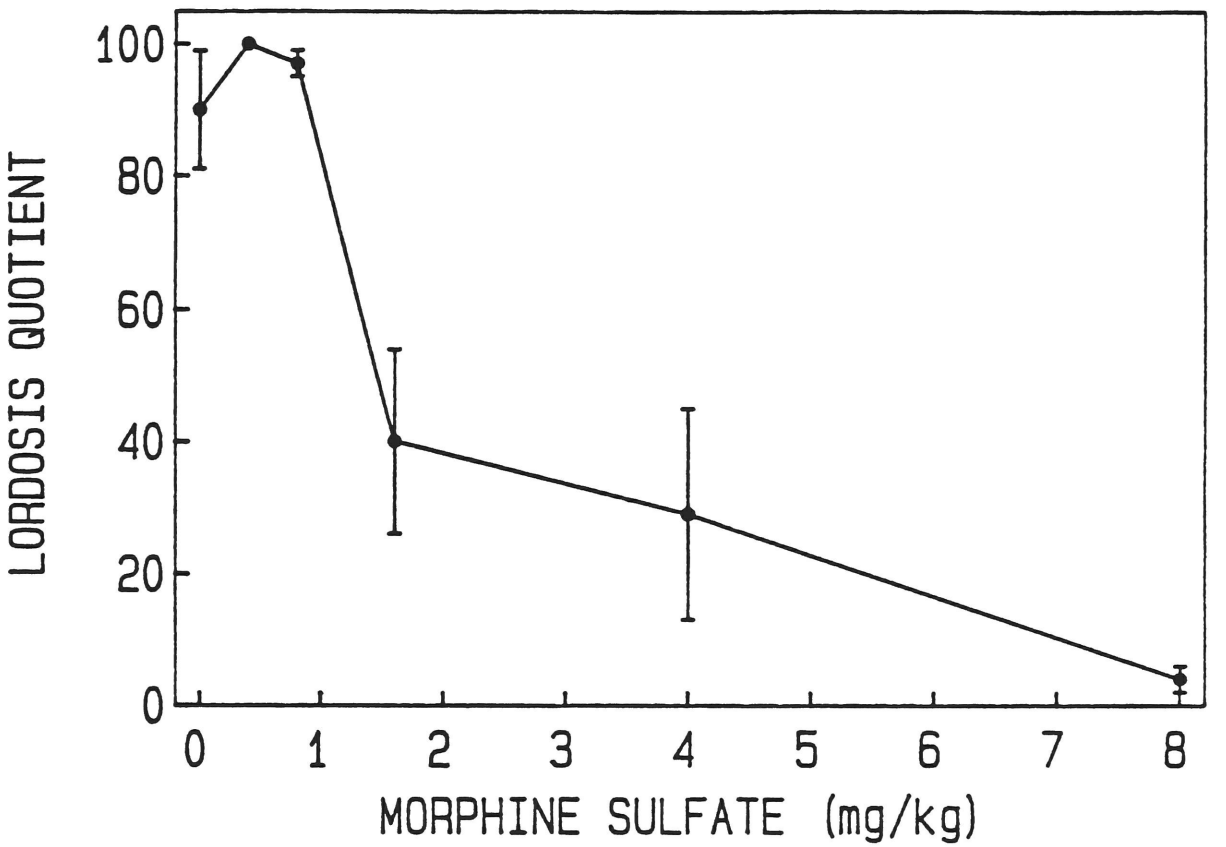


Figure 4. Morphine inhibition of lordosis.

Ovariectomized female rats were primed with estradiol benzoate (5 ug) and progesterone (500 ug, 44 hours later). Sexual behavior was tested four hours after progesterone. Rats were then injected subcutaneously with saline or morphine sulfate, and retested 10 to 20 minutes later. Values are the mean $\pm$ S.E.M. of the lordosis quotient of the second test.

Each point is the mean $\pm$ s.e.m. of 4 to 10 values.

Discussion

The opiate agonist morphine decreases lordosis behavior in estrogen primed female rats. The action appears to be specific to sexual behavior, since doses of morphine that inhibited lordosis, did not appear to grossly affect activity. The specificity of the opiate inhibition of sexual behavior has been studied more thoroughly by Weisner and Moss, 1986a, 1986c). Intraventricular administration of beta-endorphin decreased lordosis behavior without altering blood pressure, total movement, or the responsiveness to general somatosensory stimuli. Decreases in ambulation and rearing in an open field test after beta-endorphin were not correlated with decreases in sexual behavior (Weisner and Moss, 1986a). Thus, in the work presented here, paradigms have been established where opiate agonists reliably inhibit behavior, and opiate antagonists facilitate behavior. These treatments will be utilized in further studies to characterize opiate regulation of lordosis.

E. INTRACRANIAL OPIATES AND LORDOSIS

Introduction

Systemic injections of morphine and naltrexone alter lordosis in hormone primed female rats. It was of interest to localize the action of these drugs to specific areas of the brain. The behavioral actions of direct application of

opiates into areas of the brain important for lordosis were examined.

Methods

Rats were implanted with bilateral cannulae into the area above either the VMN or POA (Luine and Fischette, 1982). Rats were primed with estradiol benzoate, and sexual behavior was tested forty-four hours later. After this initial testing, rats were divided into responders and nonresponders. The inhibition of lordosis was tested in the responders, while the nonresponders were used to examine the facilitation of lordosis. In the VMN, crystalline implants of naltrexone or morphine or empty inner cannulae were inserted into the outer cannulae, and sexual behavior was tested one, two and three hours later. In the POA, saline (1 ul), morphine (2 x 2 ug) or naltrexone (2 x 3 ug or 2 x 0.1 ug) were infused into the POA. See the methods section (IX,C) for details. Sexual behavior was tested after twenty minutes and one hour. Changes in lordosis behavior after intracranial application of opiates was tested using repeated-measures analysis of variance.

Results

In the VMN, morphine inhibited sexual behavior (Figure 5), but no facilitation was seen after naltrexone. Morphine infusion (4 ug) into the POA also inhibited lordosis (Figure 6). Lordosis quotients and quality scores were decreased at both twenty minutes and one hour after

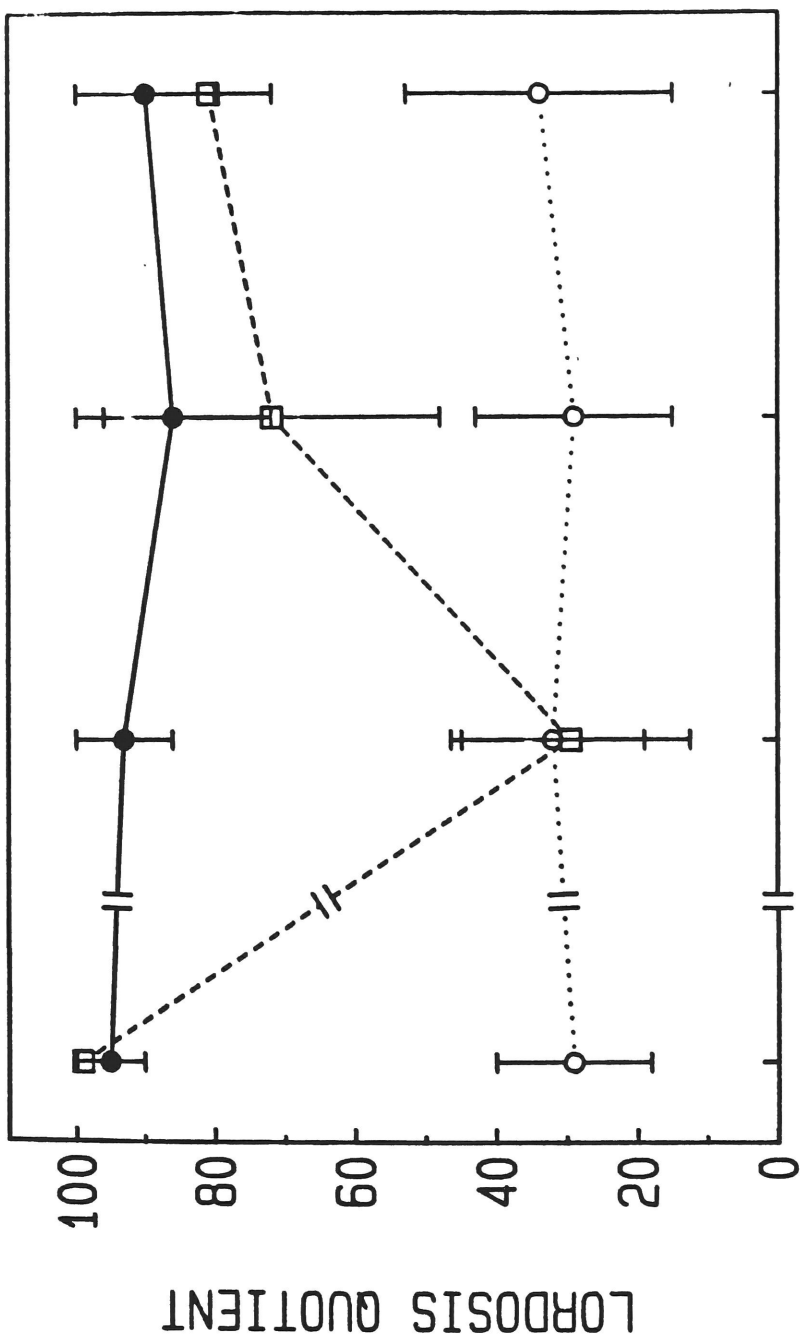


Figure 5. Effects of infusion of morphine or naltrexone in the VMN on lordosis behavior.

Ovariectomized female rats were implanted with cannulae into the VMN. Rats were primed with estradiol benzoate (5 ug), and sexual behavior was tested 44 hours later. Inner cannulae containing morphine sulfate (dashed lines) or naltrexone hydrochloride (dotted line) or empty inner cannulae (solid line) were inserted into the guide cannulae, and lordosis was tested one, two and three hours after implantation. Results are the mean $\pm$ S.E.M. of 4 to 6 animals.

Morphine sulfate inhibited lordosis one hour after implantation ($p < 0.05$). Naltrexone hydrochloride did not facilitate sexual behavior.

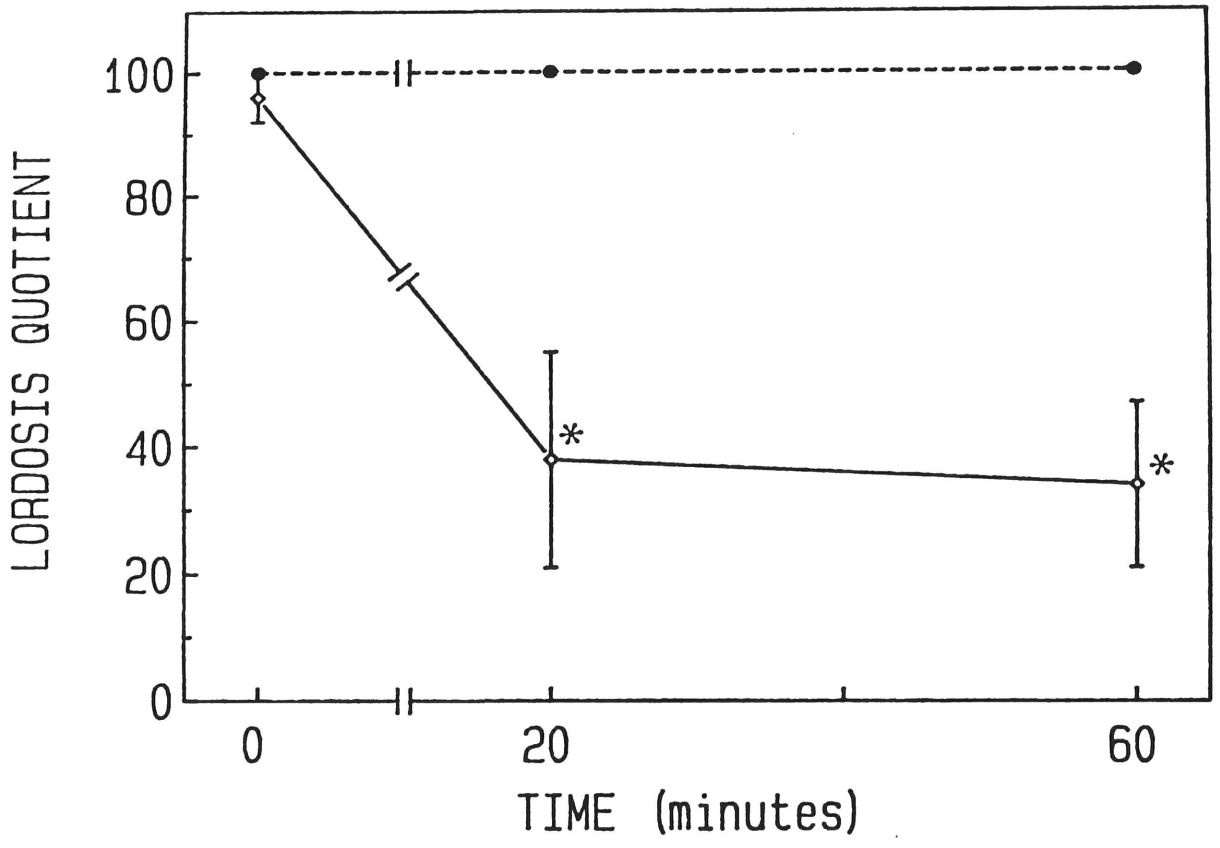


Figure 6. Inhibition of lordosis after infusion of morphine into the preoptic area.

Ovariectomized female rats were implanted with cannulae into the preoptic area. Rats were primed with estradiol benzoate (5 ug) and sexual behavior was tested 44 hours later. Saline or morphine (2 x 2 ug) was infused into the preoptic area, and sexual behavior was tested 20 and 60 minutes later. Morphine infusion is indicated by the solid line, and saline infusion by the dashed line.

* $p < 0.05$ compared to saline infusion.

Values are the mean $\pm$ s.e.m. for 3 to 5 animals.

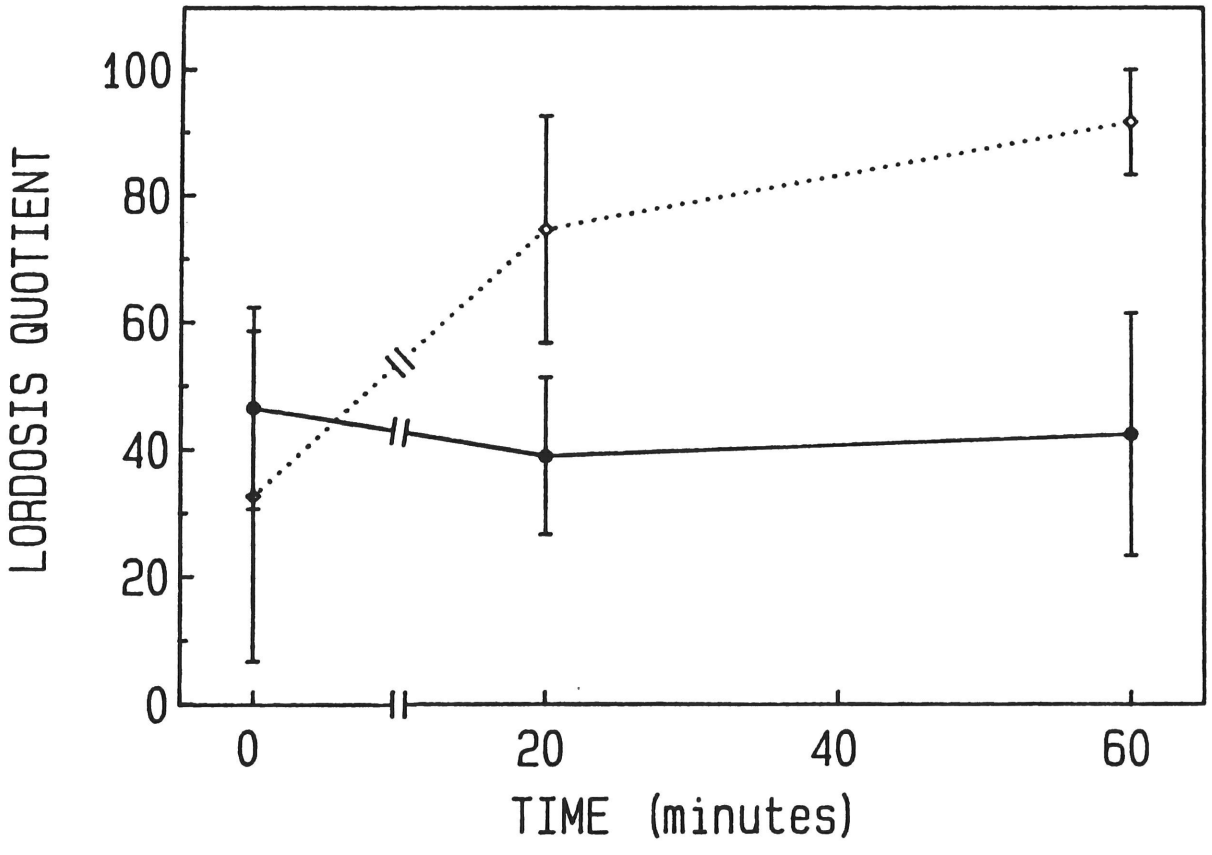


Figure 7. Facilitation of lordosis after infusion of naltrexone into the preoptic area.

Cannulae were implanted into the preoptic area of ovariectomized rats. Rats were primed with estradiol benzoate (5 ug), and sexual behavior was tested 44 hours later. Saline (solid line) or naltrexone (2 x 3 ug, dashed line) were infused into the preoptic area, and sexual behavior was tested 20 and 60 minutes later. Naltrexone increased lordosis quotients compared to saline (Repeated measures analysis of variance). Each point is the mean $\pm$ S.E.M. of 3 to 5 values.

infusion. In contrast to the VMN, naltrexone infusion into the POA facilitated behavior (Figure 7). Two different doses of naltrexone (6 ug and 0.2 ug) were used, and both doses increased lordosis behavior.

Discussion

The action of opiates in discrete areas of the brain are sufficient to modify sexual behavior. The role of two brain areas, the POA and the VMN, in the opiate regulation of behavior were examined. These two areas are important in the hormone dependent regulation of lordosis. Naltrexone infusion into the POA facilitated lordosis, while morphine in either the POA or the VMN inhibited behavior.

Sirinathsinghji (1986) also reported the inhibition of lordosis after the infusion of beta-endorphin into the preoptic area. Thus, opiates act in relevant areas of the brain to regulate the expression of lordosis.

F. CONCLUSIONS

Systemic injection of the opiate antagonist naltrexone facilitated sexual behavior in estrogen-primed female rats. The actions of naltrexone are not due to the release of adrenal progesterone, and do not appear to require protein synthesis. Infusion of naltrexone directly into the POA was sufficient to facilitate behavior.

Other investigators have also examined the facilitation of behavior by opiate antagonists. Infusion of naloxone

into the midbrain central gray (Sirinathsinghji, 1984), the medial preoptic area (Sirinathsinghji, 1986), or the subarachnoidal space around the lumbrosacral spinal cord (Lindblom, Forsberg, and Sodersten, 1986) facilitated lordosis in estrogen-primed female rats.

More recently, the role of specific subtypes of opiate receptors in the regulation of behavior has been investigated. Weisner and Moss (1986b) reported that the inhibition of lordosis by beta-endorphin was completely reversed by pretreatment with the mu_1 receptor antagonist naloxazone, but only partiallly reversed by the delta receptor antagonist ICI-154,129. Activation of high-affinity mu opiate receptors (mu_1) inhibited lordosis (Pfaus, Pendleton and Gorzalka, 1986; Pfaus and Gorzalka, 1987a). However, agonists binding to delta-receptors and low-affinity mu-receptors facilitated behavior (Pfaus, Pendleton and Gorzalka, 1986; Pfaus and Gorzalka, 1987a). Both the facilitatory and inhibitory actions of opiates were blocked by naloxone, though i.c.v. naloxone alone did not alter lordosis. The kappa-receptor agonist dynorphin 1-9 did not significantly affect lordosis (Pfaus and Gorzalka, 1987a), though another kappa agonist, leumorphin (dynorphin B-29), facilitated behavior after infusion into the VMN (Sakuma and Akaisha, 1987). Thus, studies using agonists specific for subtypes of opiate receptors show that opiate

regulation of lordosis is complex and involves both facilitation and inhibition of behavior by opiates.

The existence of both inhibitory and facilitatory actions of endogenous opiates might explain the dose and time course of naltrexone facilitated lordosis. Opiate antagonists would block both facilitatory and inhibitory actions of endogenous opiates. It may be that only with the one dose after three hours that naltrexone blocks primarily opiate inhibition of lordosis. This needs to be investigated using opiate antagonists that are more specific for the different opiate subtypes than naltrexone.

Further studies are required in order to more completely understand opiate regulation of lordosis. The role of opiate receptor subtypes in areas of the brain important for the expression of lordosis needs to be investigated. Only then can the role of the different opioid systems; beta-endorphin, the enkephalins, and the dynorphins; in the hormone-dependent regulation of lordosis be clarified.

III. ROLE OF MONOAMINERGIC SYSTEMS IN OPIATE REGULATION OF LORDOSIS

It has been established that exogenous opiates alter sexual behavior in female rats (Sirinathsinghji, 1984, 1986; Weisner and Moss, 1984, 1986a, 1986b; Allen, Renner and Luine, 1985). Neurotransmitter systems other than opiates are also known to be important in the regulation of behavior. It was possible that interactions of opiates with other neurotransmitter systems might be important for the opiate regulation of sexual behavior. As discussed briefly in the introduction, monoaminergic neurotransmitters in the brain are important for the regulation of sexual behavior. In addition, previous studies show that opiates interact with monoaminergic systems to regulate another endocrine function, luteinizing hormone secretion (Kalra and Kalra, 1983; 1984). Therefore, the next experiments examined possible monoamine-opiate interactions in regulating lordosis.

A. PARGYLINE INHIBITION OF NALTREXONE-FACILITATED LORDOSIS

Introduction

Monoamine oxidase inhibitors, which increase monoamine levels in the brain, block the facilitation of sexual behavior after estrogen and progesterone (Luine and Paden, 1982). The inhibition of sexual behavior caused by these drugs is correlated with the increase in serotonin levels,

but not with the increase in norepinephrine. It was examined if pargyline, a monoamine oxidase inhibitor that blocks progesterone-facilitated lordosis, was able to inhibit naltrexone-facilitated behavior.

Methods

Ovariectomized female rats were injected with estradiol benzoate (5 ug, s.c.), and naltrexone (3 mg/kg, forty two hours later). Two hours before naltrexone, rats were injected with pargyline (50 mg/kg, injected in a lateral tail vein), or saline. This dose of pargyline has been shown to inhibit lordosis by over 90%. Sexual behavior was tested three hours after naltrexone.

Results

As previously found, naltrexone facilitated lordosis in estrogen primed female rats (Figure 8). Pargyline blocked the facilitation of sexual behavior. Thus the increase in monoamine levels by pargyline, which blocks progesterone facilitated behavior, also antagonizes the facilitation of behavior by naltrexone.

Discussion

The pargyline inhibition of naltrexone-facilitated sexual behavior suggested that there might be important interaction between monoamines and endogenous opioid peptides in the regulation of lordosis behavior. Of particular interest was to examine opiate interactions with serotonergic systems, since serotonin has been shown to be

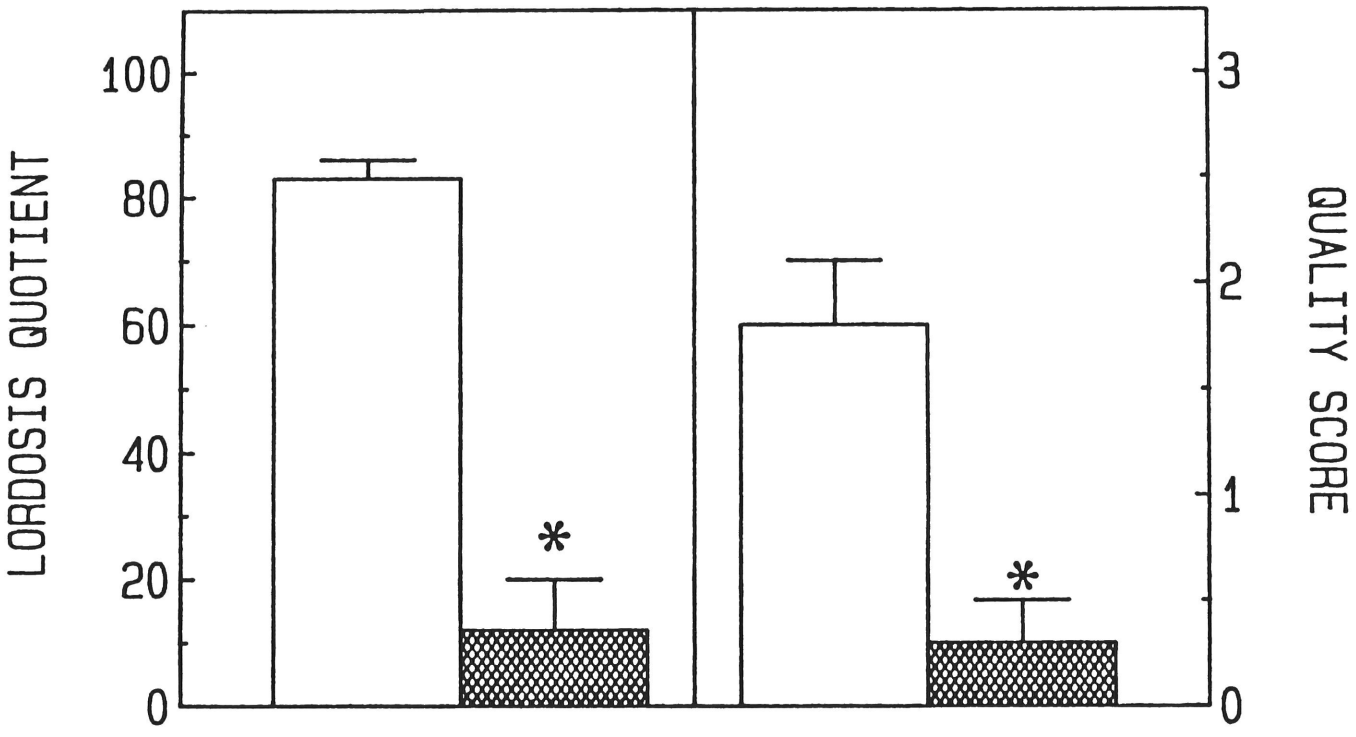


Figure 8. Pargyline inhibition of naltrexone-facilitated lordosis.

Ovariectomized female rats were injected with estradiol benzoate (5 ug) and naltrexone (3 mg/kg, 42 hours later). Two hours before naltrexone, rats were injected with saline (open bars) or pargyline (50 mg/kg, cross-hatched bars), and sexual behavior was tested three hours after naltrexone. Values are the mean $\pm$ s.e.m. for three to five rats.

* $p < 0.05$ compared to saline treatment using Mann-Whitney U-test.

important for the hormone-dependent regulation of lordosis. Naltrexone might be controlling the release of monoamines in the preoptic area and hypothalamus, which may then act directly to facilitate behavior (Figure 9). However, monoaminergic pathways could be parallel to opiate systems, and the inhibition by pargyline could simply be over-riding the facilitation by naltrexone.

B. FAILURE OF MORPHINE TO INHIBIT LORDOSIS AFTER SEROTONERGIC LESIONS

Introduction

In preliminary work, Moreines and McEwen, (personal communication), studied opiate regulation of sexual behavior in rats in which hypothalamic serotonergic terminals were lesioned by the selective serotonergic neurotoxin 5,7-dihydroxytryptamine. This lesion results in high levels of lordosis responding in rats after doses of estrogen that are normally subthreshold (Luine, Frankfurt, Rainbow, Biegon and Azmitia, 1983). Using very low doses of estrogen, that alone did not facilitate sexual behavior, Moreines and McEwen showed that naltrexone was not able to facilitate lordosis, while progesterone was able to facilitate behavior. Their results suggest that an intact serotonergic system is necessary for the facilitation of behavior by naltrexone.

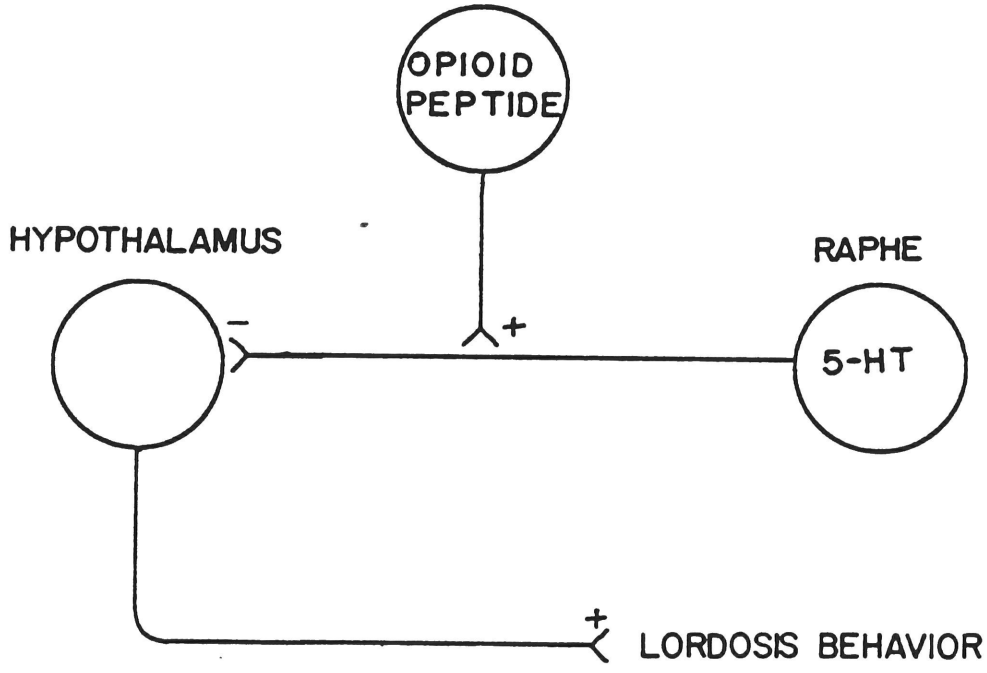


Figure 9. Proposed model for opiate regulation of lordosis.

An intact serotonergic system may be required for the inhibition of behavior by morphine. If opiates alter serotonin release in the hypothalamus and preoptic area, and this is the mechanism for opiate regulation of behavior, lesioning serotonergic terminals in the hypothalamus would disconnect the opiate input from neurons in the hypothalamus important for the expression of lordosis. Therefore, the ability of morphine to inhibit behavior in rats with hypothalamic serotonergic lesions was tested.

Methods

Hypothalamic serotonergic terminals were lesioned with the selective neurotoxin 5,7-dihydroxytryptamine as described in the methods. This lesion decreases serotonin levels in the POA and VMN by 90% at 7 days after the lesion, and the decrease is still present 26 days later (Frankfurt, Renner, Azmitia and Luine, 1985). The lesion produces high levels of lordosis after low doses of estrogen. Facilitated behavior is seen at one week after the lesion, and continues for up to 8 weeks, so there is a long period of time in which to investigate opiate regulation of behavior.

Lesioned rats were primed with 5 ug estradiol benzoate. Sham lesioned rats were injected with 5 ug estradiol benzoate and progesterone (500 ug 44 hours later). All rats were tested for sexual behavior 48 hours after estrogen. After initial testing, all rats were injected with morphine sulfate (2 mg/kg). This dose inhibits lordosis responding

by more that 50 percent in estrogen-progesterone treated rats (Figure 4). Sexual behavior was tested 10 to 20 minutes after morphine.

Results

The hormone treatments facilitated sexual behavior in both the lesioned and the sham lesioned rats (Figure 10). Confirming the results in Section II.,D, morphine sulfate inhibited sexual behavior in estrogen and progesterone primed sham lesioned rats by over fifty percent. However, this same dose of morphine did not significantly decrease lordosis quotients in the rats with 5,7-DHT lesions.

Discussion

The observation that morphine and naltrexone are not able to respectively inhibit or facilitate female sexual behavior in rats with hypothalamic serotonergic lesions, suggested that an intact hypothalamic serotonergic system is necessary for opiate regulation of behavior. One possibility was that naltrexone may be facilitating sexual behavior by altering serotonin turnover in areas of the brain important for the regulation of sexual behavior. Previous work by other investigators has shown that opiates can regulate the rate of serotonin turnover in the brain. Chronic treatment with morphine has been reported to increase the activity of tryptophan hydroxylase in the midbrain (Azmitia, Hess and Reis, 1970) and the septal area (Knapp and Mandel, 1972). Acute morphine increases the rate

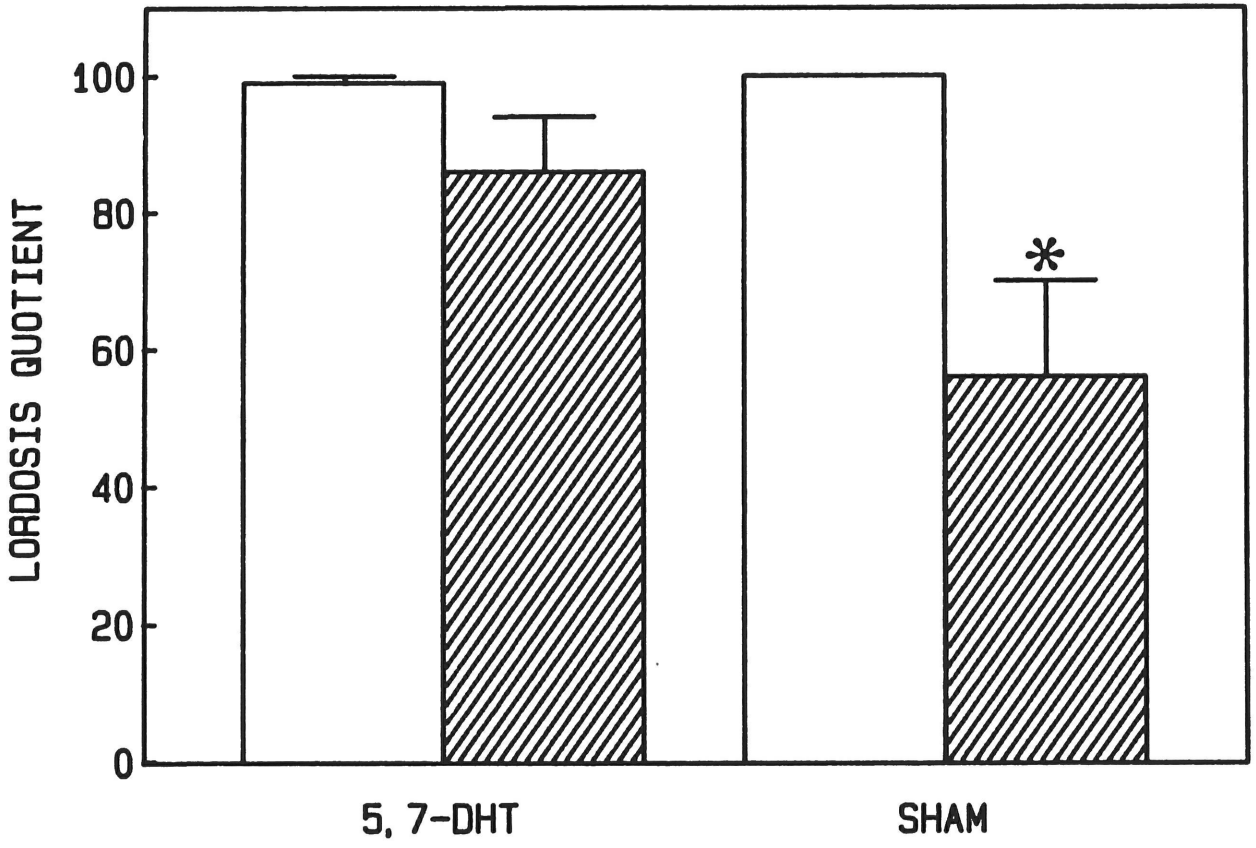


Figure 10. Morphine inhibition of lordosis after hypothalamic serotonergic lesions.

Hypothalamic serotonergic terminals were lesioned with the selective neurotoxin 5,7-dihydroxytryptamine (5,7-DHT). Sham lesioned rats were infused with vehicle. Lesioned rats were primed with 5 ug estradiol benzoate. Sham lesioned rats were primed with both estradiol benzoate and progesterone (500 ug). Sexual behavior was tested 48 hours after estrogen (open bars). All rats were injected with morphine sulfate (2 mg/kg), and sexual behavior was tested again 10 to 20 minutes later (striped bars). Values are the mean $\pm$ s.e.m. of six rats.

* $p < 0.05$ compared the the behavioral test before morphine treatment.

of serotonin synthesis and levels of 5-HIAA, the major metabolite of serotonin (Spampinato, Esposito, Romandini and Samanin, 1985; Johnston and Moore, 1983). These effects of morphine are blocked by the opiate antagonist naloxone. Opiates also alter serotonin turnover in some areas of the brain important for the regulation of luteinizing hormone secretion; many of these same areas being important for the regulation of sexual behavior. Morphine increased serotonin turnover in the preoptic area and the arcuate nucleus (Johnston and Moore, 1983; Johnson and Crowley, 1984), and the effects of morphine were blocked by naloxone. But opiate regulation of serotonin turnover has not been studied with the same paradigms used to study opiate regulation of behavior.

IV. METHODS TO MEASURE MONOAMINE TURNOVER

One way to investigate the role of serotonergic systems in opiate regulation of lordosis is to examine the effects of opiates on the release of serotonin in the brain. Biochemical methods have been developed to estimate neurotransmitter release by measuring their rate of synthesis or degradation. These methods to estimate monoamine turnover have been previously described and critiqued (Costa and Neff, 1970; Weiner, 1974), and a short synopsis and critique of the methods follows.

The monoaminergic neurotransmitters are synthesized from the essential amino acids tyrosine and tryptophan (Figure 11,12). The first step is the hydroxylation of the amino acids by tyrosine hydroxylase (Levitt, Spector, Sjoerdsma and Udenfriend, 1965; Udenfriend, 1966; Nagatsu, Levitt and Udenfriend, 1969) or tryptophan hydroxylase. Tryptophan hydroxylase and tyrosine hydroxylase (Nagatsu, Levitt and Udenfriend, 1964; Levitt, Spector, Sjoerdsma and Udenfriend, 1965) are the rate limiting enzymes in the synthesis of serotonin and the catecholamines. While tyrosine hydroxylase is subject to significant end-product inhibition by catecholamines (Neff and Costa, 1966; 1968; Spector, Gordon, Sjoerdsma and Udenfriend, 1967; Ngai, Neff and Costa, 1968, Lin, Neff, Ngai and Costa, 1969), tryptophan hydroxylase is not significantly inhibited by increased levels of serotonin (Lin, Neff, Ngai and Costa,

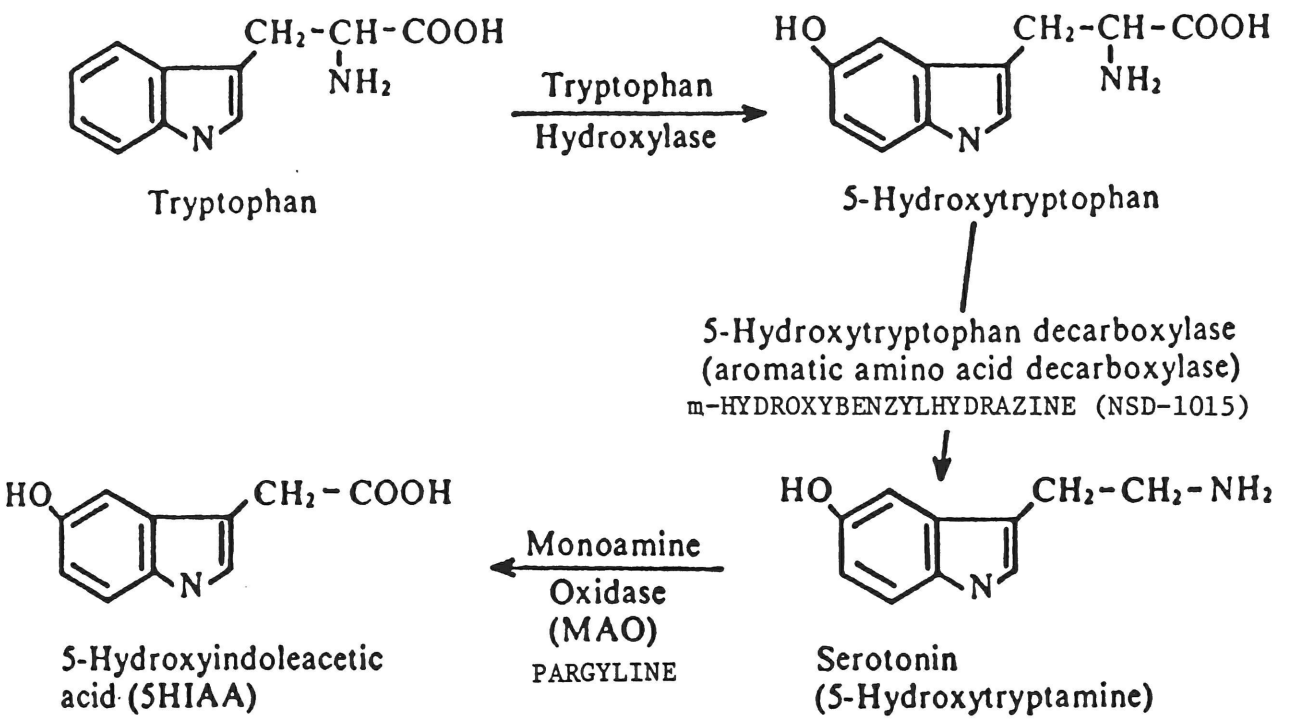


Figure 11. Serotonin Synthesis and Metabolism.
(From Cooper, Bloom and Roth, 1978)

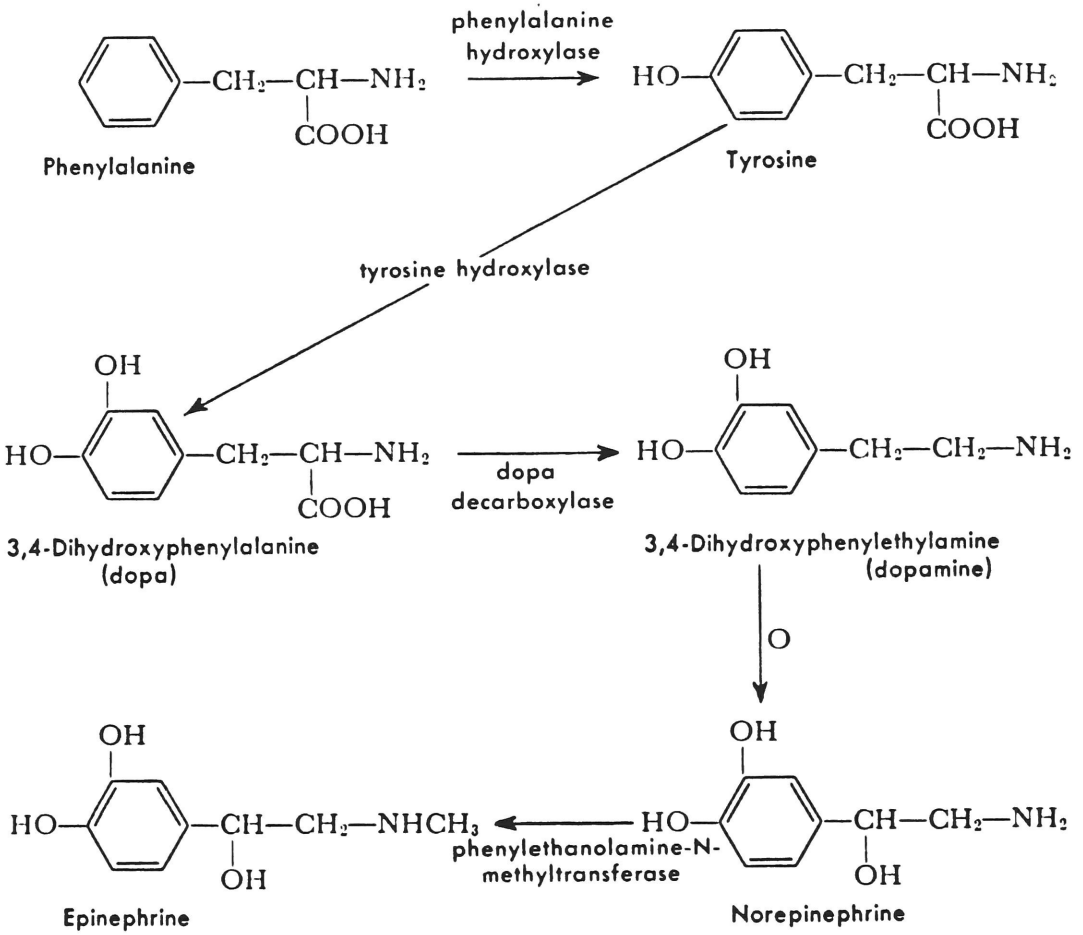


Figure 12. Catecholamine synthesis and metabolism.
(From Turner and Bagnara, General Endocrinology, 1976)

1969; Millard, Costa and Gal, 1972). Both hydroxyamino acids are decarboxylated by aromatic amino acid decarboxylase (Sourkes, 1966) to form dopamine or 5-hydroxytryptamine (serotonin). Norepinephrine is subsequently formed by the beta-hydroxylation of dopamine (Levin, Levenberg and Kaufman, 1960). Serotonin is inactivated by monoamine oxidase to form 5-hydroxyindole acetic acid. Norepinephrine and dopamine are also metabolized by monoamine oxidase, in addition to being inactivated by catecholamine-ortho-methyltransferase (Axelrod and Tomchick, 1958).

Initial methods to measure monoamine turnover involved injecting rats with radiolabeled neurotransmitters or their precursors, and measuring changes in levels and specific activity of the radiolabeled neurotransmitters over time (Spector, Gordon, Sjoerdsma and Udenfriend, 1967; Lin, Neff, Ngai and Costa, 1969). Mathematical models were developed to estimate the rate of monoamine synthesis. The amount of radiolabeled tryptophan or tyrosine were small compared to endogenous levels of these amino acids, so the treatment with exogenous amino acids did not dramatically alter monoamine metabolism. However, the experiments are difficult, requiring the purification of the amino acid and the neurotransmitter, and the measuring of concentration and specific activity. The other major problem with these methods for the present experiments is the difficulty in

incorporating a high level of radiolabel into the neurotransmitter. I was interested in measuring monoamine turnover in discrete brain nuclei of the hypothalamus that have been implicated in the regulation of female sexual behavior. Using the same level of incorporation as reported by others (Lin, Neff, Ngai and Costa, 1969; Spector, Gordon, Sjoerdsma and Udenfriend, 1965) less than one count per minute would be incorporated into serotonin or norepinephrine in most of the areas of interest.

More recently, methods to estimate the release of neurotransmitters have been developed that measure levels of neurotransmitters or their precursors or metabolites, in some cases after pharmacological treatments that inhibit steps in monoamine metabolism. These methods are technically easier, since they do not require determining the specific activity of the neurotransmitters. However, they usually dramatically alter monoaminergic function in the brain. Different methods have been developed for serotonin and the catecholamines, norepinephrine and dopamine.

A. CATECHOLAMINE TURNOVER

The standard method for the estimation of catecholamine release is to measure the decrease in catecholamine levels after inhibiting the synthesis of catecholamines with alpha-methyl-p-tyrosine (AMPT, Brodie, Costa, Dlabac, Neff

and Smookler, 1966). AMPT is a potent inhibitor of tyrosine hydroxylase, the enzyme which converts tyrosine into DOPA (Levitt, Spector, Sjoerdsma, and Udenfriend, 1965). Tyrosine hydroxylase is the rate limiting enzyme of catecholamine synthesis (Levitt, Spector, Sjoerdsma, and Udenfriend, 1965). AMPT acts rapidly and completely to inhibit tyrosine hydroxylase (Spector, Gordon, Sjoerdsma and Udenfriend, 1965; Brodie, Costa, Dlabac, Neff and Smookler, 1966), but does not cause the release of catecholamines or alter their metabolism (Spector, Gordon, Sjoerdsma and Udenfriend, 1965). As synthesis is inhibited, levels of catecholamines decrease over time at a rate that is proportional to the level of catecholamine.

$$d[CA]/dt = - k [CA]$$

Integrating this expression.

$$[CA] = [CA]_o \, e^{-kt},$$

where $[CA]_o$ is the initial levels.

Converting to natural logarithm results in

$$\ln [CA] = \ln [CA]_o - kt$$

where k is the slope of the decrease in ln [CA] after inhibition of synthesis by AMPT.

The rate of catecholamine turnover is equal to k x $[CA]_o$, where k is the rate constant of catecholamine decrease, and $[CA]_o$ is the normal amine concentration. This method has been used to estimate catecholamine turnover in both the whole brain and in discrete brain areas, and has

also been able to detect changes in turnover after lesions (Anden, Corrodi, Dahlstrom, Fuxe and Hokfelt, 1966; Anden, Corrodi, Fuxe and Ungerstedt, 1971), hormones (Crowley, O'Donohue, Wachslicht and Jacobowitz, 1978; Crowley, 1982; Gunnet, Lookingland and Moore, 1986; Renner, Krey and Luine, 1987), or drugs (Anden, Corrodi, Fuxe and Ungerstedt, 1971).

B. SEROTONIN TURNOVER

Several different methods have been applied to estimate serotonin turnover in the brain. Unlike with tyrosine hydroxylase, there is no drug that rapidly, completely and selectively inhibits tryptophan hydroxylase. Para-chlorophenylalanine inhibits tryptophan hydroxylase, but it is not suitable for use in turnover experiments. Three other biochemical methods have been developed to estimate serotonin release in the brain. Studies which have used more than one method to estimate serotonin turnover have found that, in most cases, these different methods give qualitatively similar results (Van Loon, Shum and Sole, 1981). Some contradictory evidence has been recently advanced (Shannon, Gunnet and Moore, 1986), and will be discussed more completely in Section IV,B,d.

a. Levels of 5-hydroxyindoleacetic acid

Levels of serotonin and its principal metabolite, 5-hydroxyindole acetic acid (5-HIAA), can be compared between experimental groups. However, changes in levels of

serotonin are not reliable indicators of changes in serotonin turnover in the brain. Levels of serotonin are not always altered by treatments which change the rate of monoamine turnover, such as some treatments with hormones (Johnson and Crowley, 1986) or drugs (Johnson and Crowley, 1984). Treatment with monoamine oxidase inhibitors increases serotonin levels in the brain, but does not change the rate of synthesis as measured using radiochemical methods (Lin, Neff, Ngai and Costa, 1969; Millard, Costa and Gal, 1972). These results also suggest that there is a lack of significant feedback regulation in serotonergic systems. Changes in levels of 5-HIAA have also been used to indicate changes in serotonin metabolism or turnover (Moir, 1971), though Kuhn and colleagues (1986) have suggested that 5-HIAA levels primarily reflect monoamine oxidase activity, not the release of serotonin. While this method is the easiest and involves the least manipulation of experimental animals, it does not appear to be as sensitive as other methods. Small changes in serotonin metabolism may not be reflected by measurable changes in 5-HIAA levels (Renner, Krey and Luine, 1987). In addition, changes in levels of 5-HIAA may be due to actions of the experimental treatment on aspects of serotonin function other than serotonin metabolism. For example, ovariectomy decreases the rate of efflux of 5-HIAA from the brain (Duval and Mignot, 1985).

b. Pargyline method

Serotonin turnover has also been estimated by measuring the increase in levels of serotonin after pargyline (Tozer, Neff and Brodie, 1966). Pargyline rapidly and completely inhibits monoamine oxidase, the primary degradative enzyme for serotonin. Serotonin levels increase linearly for approximately fifteen to sixty minutes, depending on the area of the brain examined (Johnson and Crowley, 1983; 1986; Renner, Allen and Luine, 1986). The rate of the increase has been used as an index of serotonin turnover. Pargyline does not alter the rate of serotonin synthesis as measured by the rate of conversion of ^{14}C-tryptophan to radioactive serotonin (Lin, Neff, Ngai and Costa, 1969; Millard, Costa and Gal, 1972).

The major methodological problem with this method is the presence of measurable levels of serotonin in the brain before pargyline treatment. The estimation of serotonin turnover is the difference between initial levels of serotonin and the levels of serotonin after pargyline. The increase in serotonin levels after pargyline is linear for short periods of time. The rate of serotonin increase was analyzed by linear regression analysis and used as an index of serotonin turnover. The slopes of the increase in serotonin over time can be compared between groups (Renner, Allen and Luine, 1986).

While pargyline inhibits the degradation of both catecholamine and serotonin by monoamine oxidase, this method is only effective in measuring serotonin turnover. The increase in levels of norepinephrine and dopamine after pargyline inhibit tyrosine hydroxylase (Neff and Costa, 1966; 1968; Spector, Gordon, Sjoerdsma and Udenfriend, 1967; Ngai, Neff and Costa, 1968, Lin, Neff, Ngai and Costa, 1969), the rate-limiting enzyme in the synthesis of catecholamines. Catecholamines are also metabolized by catecholamine o-methyl transferase (Axelrod and Tomchick, 1958), so that pargyline would not completely inhibit degradation.

c. Aromatic amino acid decarboxylase method

The last method commonly used for the measurement of serotonin turnover is to measure the increase in levels of 5-hydroxytryptophan after inhibiting aromatic amino acid decarboxylase (Figure 11). Levels of 5-hydroxytryptophan increase linearly over short periods of time, and have been used as an index of tryptophan hydroxylase activity (Shannon, Gunnet and Moore, 1986; King, Steger and Morgan, 1986). Since levels of 5-hydroxytryptophan are normally below the limit of detection of most HPLC methods, usually the only measurement is of levels of 5-HTP at a set time after administration of an AADC inhibitor. One problem with this method is that inhibition of AADC dramatically

increases levels of 5-HTP over its usual low levels, and the effect of this increase in 5-HTP levels on serotonin turnover is not known. 5-HTP also crosses the blood-brain barrier, so increases in levels of 5-HTP most likely underestimate the rate of synthesis and the estimate of serotonin turnover, though they are still a good index of serotonin synthesis.

d. General critique of turnover methods

All of the non-steady state methods for measuring monoamine turnover have significant drawbacks. The methods assume that there is a single pool of neurotransmitter that is turning over at a constant rate. This was more of a problem with earlier studies which measured neurotransmitter in the whole brain. In many cases, the tissue that was examined included cell bodies, fibers of passage, and axon terminals. This is less of a problem with the turnover experiments presented here. All tissue in these experiments was sampled using the Palkovits "punch technique" (1973), which allows the reliable and consistent dissection of small areas of the brain. By using this method, we have restricted the cellular location and the functional use of the neurotransmitter. In the areas sampled, serotonergic cells bodies are only found in the dorsomedial nucleus of the hypothalamus, while dopaminergic neurons are found in the arcuate nucleus. In all other sampled areas, the

monoamines are contained in axon terminals or fibers of passage.

These non-steady state methods of measuring monoamine turnover perturb the very systems we are attempting to measure. We are measuring the changes in levels of neurotransmitters or their precursors or metabolites. The increases or decreases in these neuroactive molecules are probably further altering neurotransmitter turnover. In addition, many of these pharmacological treatments are not specific to one neurotransmitter. Inhibiting aromatic amino acid decarboxylase blocks the synthesis of both serotonin and the catecholamines, while pargyline blocks their degradation. If one monoamine regulates the release of another (Moir, 1971), monoamine turnover may be altered by the method of measuring turnover.

The other major problem with these experiments is that the quantity we are most interested in is the amount of neurotransmitter that is released from the post-synaptic axon terminal, crosses the synaptic cleft, and binds to receptors on the post-synaptic membrane, resulting in some physiological effect. What these experiments more precisely measure is the total synthesis and degradation of the monoamine, without discriminating between monoamine that is being released and monoamine metabolized within the presynaptic cell. There is some evidence to suggest that most of the serotonin that is synthesized in a neuron is

metabolized within that neuron without having been released (Kuhn, Wolf and Youdin, 1986).

Recently, Moore and colleagues have questioned the validity of the "pargyline method" as an index of serotonin turnover in the brain (Shannon, Gunnet and Moore, 1986). In their experiments, the dorsal raphe nucleus was electrically stimulated. Five different methods were then used to measure serotonin turnover: levels of serotonin and 5-HIAA without any pharmacological manipulations, the increase in serotonin and the decrease in 5-HIAA after pargyline, and levels of 5-HTP after the AADC inhibitor NSD-1015. They examined four discrete areas of the brain which receive projections from the dorsal raphe (Azmitia, 1978), the nucleus accumbens, the amygdala, the suprachiasmatic nucleus, and the dorsomedial nucleus of the hypothalamus. While there were some differences among areas, the overall effect of dorsal raphe stimulation was an increase in the levels of 5-HIAA and the ratio of 5-HIAA to serotonin in non-manipulated rats, and an increase in levels of 5-HTP after NSD-1015. No change in serotonin turnover after stimulation was detected using the pargyline method, measuring either the increase in serotonin or the decrease in 5-HIAA over time. One consideration which limits the general applicability of their findings was that they measured changes in turnover after stimulation of the dorsal raphe nucleus. Many changes in monoamine turnover after

hormone or pharmacological treatments are restricted to discrete areas of the brain, suggesting that an important site for the regulation of monoamine turnover is at the level of the terminals, not the cell bodies. Other studies have shown a good correlation between all these methods to detect changes in monoamine turnover (Van Loon, Shum and Sole, 1981).

An important point is that all of these methods do not measure the amount of neurotransmitter that is released from terminals. Instead we are measuring the total synthesis or degradation of the monoamine. In most cases though, we are able to examine changes in relative rates of monoamine turnover and compare the effects of different treatments.

V. OPIATE REGULATION OF MONOAMINE TURNOVER

The next series of experiments was designed to examine the effects of opiates on monoamine turnover in areas of the brain important for the regulation of lordosis. Two major paradigms will be used. Estrogen primed female rats were injected with naltrexone. Monoamine turnover was measured at a time when naltrexone would facilitate behavior. The second group examined changes in monoamine turnover after morphine administration to estrogen and progesterone treated rats, at a time when morphine would inhibit the facilitation of lordosis by the hormones.

A. OPIATE REGULATION OF CATECHOLAMINE TURNOVER

Methods

Female rats were ovariectomized, and 5 days later were injected with estradiol benzoate (5 ug, s.c.). Forty-four hours later rats were injected with saline or naltrexone (3 mg/kg, s.c.). Rats were sacrificed three hours later. As a measure of initial levels of catecholamines, one group of rats was injected with saline one hour before decapitation. The other rats were injected with alpha-methyl-para-tyrosine methyl ester hydrochloride (400 mg/kg free base) either one or two hours before sacrifice. This dose of AMPT rapidly and completely inhibits tyrosine hydroxylase. Catecholamine levels decrease exponentially after the inhibition of synthesis. Levels of norepinephrine and dopamine were

measured in brain nuclei by HPLC with electrochemical detection. Catecholamine levels were measured in the medial preoptic area (POA), lateral portion of the ventromedial nucleus (VMN), anterior hypothalamus (AH), dorsal medial nucleus (DMN), and midbrain central gray (MCG).

Results

Dopamine levels were not altered by naltrexone (Table 2). Naltrexone increased levels of norepinephrine in the POA (Table 3). This is indicative of a change in norepinephrine turnover, but we are not able to determine the direction of the change in turnover. No differences in catecholamine levels were detected in the other brain regions examined.

Changes in catecholamine turnover were estimated by measuring the rate of decline in catecholamine levels after blocking synthesis with the tyrosine hydroxylase inhibitor AMPT (Table 2,3). Naltrexone did not alter the rate of decline of dopamine after the inhibition of catecholamine synthesis, or the turnover rate. In the POA, the rate of decline of norepinephrine and the turnover rate were increased by naltrexone.

Discussion

Dopamine turnover was not altered by naltrexone in the areas of the brain that were examined. Naltrexone only altered noradrenergic systems in the POA. Levels of norepinephrine and the rate of norepinephrine decline were

Table 2. Effects of naltrexone on dopamine levels and turnover.

AREA	TREATMENT	N	C_o (PG/UG)	K (HR^{-1})	K (PG/UG/HR)
SCN	EB	20	2.50 ± .26	.702 ± .148	1.7 ± .5
	EB + NTX	21	1.84 ± .26	.538 ± .119	.99 ± .31
DMN	EB	20	1.62 ± .17	.475 ± .079	.77 ± .22
	EB + NTX	20	1.99 ± .21	.497 ± .100	.99 ± .29
ARC-ME	EB	18	23.5 ± 1.3	.523 ± .063	12.3 ± 1.7
	EB + NTX	19	29.1 ± 2.5	.561 ± .089	16.3 ± 2.9
DBB	EB	21	2.40 ± .64	.640 ± .252	1.5 ± .7
	EB + NTX	18	1.75 ± .27	.787 ± .243	1.4 ± .6
LS	EB	19	2.32 ± .43	.402 ± .189	.93 ± .50
	EB + NTX	21	2.18 ± .46	.385 ± .176	.82 ± .42
AH	EB	21	2.07 ± .32	.515 ± .117	1.1 ± .3
	EB + NTX	21	2.28 ± .21	.516 ± .143	1.2 ± .4
POA	EB	20	2.98 ± .47	.811 ± .178	2.4 ± .7
	EB + NTX	20	2.98 ± .26	.674 ± .152	2.0 ± .6
PVE-1	EB	21	3.70 ± .27	.476 ± .077	1.8 ± .2
	EB + NTX	21	3.56 ± .13	.542 ± .098	1.9 ± .2
PVE-2	EB	21	4.88 ± .46	.528 ± .053	2.6 ± .4
	EB + NTX	21	4.73 ± .61	.472 ± .083	2.2 ± .5

Ovariectomized female rats were injected with 5 ug estradiol benzoate, and 44 hours later with saline or naltrexone (3 mg/kg). Rats were sacrificed three hours later. As a measure of initial levels of dopamine, one group of rats was injected with saline one hour before sacrifice. The other rats were injected with alpha-methyl-p-tyrosine methylester (400 mg/kg free base) one or two hours before decapitation. Levels of dopamine were measured by HPLC. The rate constant, k, was estimated by least-squares linear regression of the slope of the decrease in log[dopamine]. The turnover rate, K, was the rate constant multiplied by the initial concentration of dopamine. Results are the mean $\pm$ S.E.M., n = 16 to 21.

The areas that were examined were the suprachiasmatic nucleus (SCN), dorsomedial nucleus (DMN), arcuate nucleus-median eminence (ARC-ME), the diagonal bands of Broca (DBB), lateral septum (LS), anterior hypothalamus (AH), ventromedial nucleus (VMN), medial preoptic area (POA), and the periventricular nucleus at the level of the preoptic area (PVE-1) and the level of the anterior hypothalamus (PVE-2).

Table 3. Effects of naltrexone on norepinephrine levels and turnover.

AREA	TREATMENT	N	C_o (PG/UG)	K (HR^{-1})	K (PG/UG/HR)
VMN	EB	20	16.7 ± 1.3	.152 ± .073	2.5 ± 1.3
	EB + NTX	20	15.8 ± 1.2	.136 ± .070	2.2 ± 1.2
SCN	EB	20	29.0 ± 2.6	.275 ± .090	8.0 ± 2.8
	EB + NTX	20	24.6 ± 1.7	.284 ± .054	7.0 ± 1.5
DMN	EB	19	26.8 ± 2.5	.197 ± .092	5.3 ± 2.6
	EB + NTX	15	22.3 ± 3.3	.143 ± .148	3.2 ± 3.6
ARC-ME	EB	17	20.7 ± 1.3	.273 ± .065	5.7 ± 1.5
	EB + NTX	21	19.3 ± 1.2	.243 ± .043	4.7 ± .9
DBB	EB	16	22.8 ± 1.4	.139 ± .043	3.2 ± 1.1
	EB + NTX	20	26.6 ± 1.4	.141 ± .071	3.7 ± 2.0
LS	EB	20	2.23 ± .30	.069 ± .087	.15 ± .21
	EB + NTX	20	2.05 ± .34	.126 ± .129	.26 ± .28
AH	EB	20	20.5 ± 1.8	.228 ± .060	4.7 ± 1.3
	EB + NTX	21	19.0 ± .8	.224 ± .059	4.2 ± 1.2
POA	EB	20	54.3 ± 3.6	.058 ± 057+	3.2 ± 3.3
	EB + NTX	21	65.7 ± 2.0*	.287 ± .053*	18.8 ± 3.7*
PVE-1	EB	21	37.2 ± 1.2	.207 ± .040	7.7 ± 1.5
	EB + NTX	21	34.0 ± 1.5	.301 ± .049	10.2 ± 1.6
PVE-2	EB	19	39.4 ± 3.3	.230 ± .045	9.1 ± 1.8
	EB + NTX	21	42.1 ± 5.0	.257 ± .078	10.8 ± 3.3

Ovariectomized female rats were injected with 5 ug estradiol benzoate, and 44 hours later with saline or naltresone (3 mg/kg). Rats were sacrificed three hours later. As a measure of initial levels of norepinephrine, one group of rats was injected with saline one hour before sacrifice. The other rats were injected with alpha-methyl-p-tyrosine methylester (400 mg/kg free base) one or two hours before decapitation. Levels of norepinephrine were measured by HPLC. These rats were the same that were used for the estimation of dopamine turnover. The rate constant and turnover rate were determined. The abbreviations are the same as in Table 1.

* $p < 0.05$ compared to EB treatment.

Values are the mean ± s.e.m. The determinations of turnover rate are from 18 to 21 animals.

both increased by naltrexone. This method for estimating catecholamine synthesis assumes a steady-state condition, that is where catecholamine synthesis equals catecholamine degradation. The change in norepinephrine levels by naltrexone indicates that we may not be at a steady-state. It is possible that a new steady-state has been achieved at a higher level of norepinephrine, but we can not determine if this is so. The increase in the rate constant suggests that naltrexone is increasing the rate of norepinephrine turnover, but this needs to be interpreted with caution. An increase in norepinephrine turnover would be consistent with the work of other investigators suggesting that opiate regulation of norepinephrine release in the POA is important in the regulation of LHRH release (Kalra and Kalra, 1983, 1984).

B. OPIATE REGULATION OF SEROTONIN TURNOVER

Different methods have been developed to estimate serotonin turnover in the brain. In order to more completely characterize and understand opiate regulation of serotonin turnover, we used three different methods to estimate turnover.

a. Pargyline method

Methods

Ovariectomized female rats were injected with estradiol benzoate (5 ug). 44 hours later saline or naltrexone (3

mg/kg, s.c.) was injected and the rats were sacrificed three hours later. Twenty minutes before decapitation, rats were injected with saline or pargyline (75 mg/kg, i.p.), an inhibitor of monoamine oxidase. Levels of serotonin in the brain increase linearly during this period of time. Discrete areas of the brain were dissected using the Palkovitz (1973) "punch" technique, and levels of serotonin were measured by HPLC with electrochemical detection. The rate of increase of serotonin levels was determined by linear regression analysis, and used as an index of serotonin turnover.

Results

Levels of serotonin in brain areas were measured without pargyline treatment. Naltrexone increased levels of serotonin in the POA compared to saline treatment (Figure 13). Levels of serotonin were not altered by naltrexone in other areas of the brain. This change in serotonin levels is indicative of a change in serotonin turnover in the POA, but it is not possible to determine if the turnover rate of serotonin is increasing or decreasing. Possible changes in serotonin turnover can be examined by measuring the rate of increase in serotonin levels after pargyline.

In the POA, pargyline treatment increased levels of serotonin in saline treated rats (Figure 14). Naltrexone abolished the increase in serotonin levels after pargyline

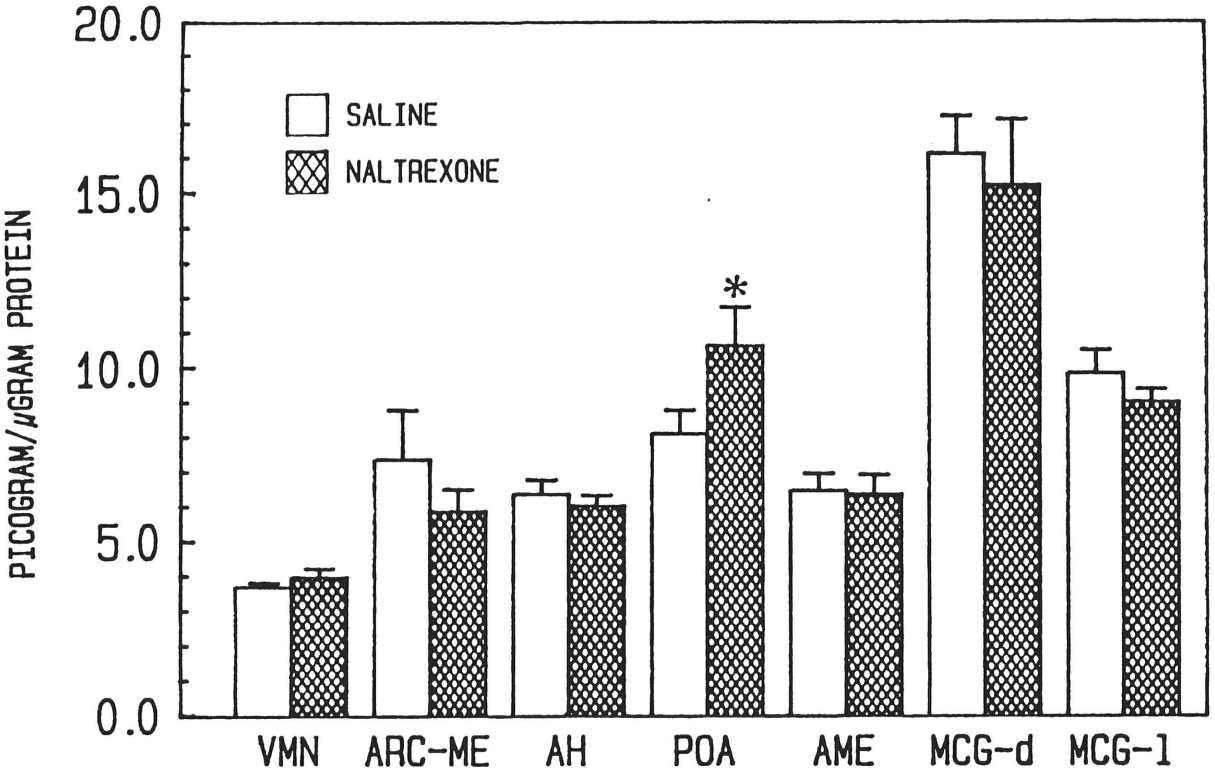


Figure 13. Effects of naltrexone on serotonin levels in estrogen-primed female rats.

Ovariectomized female rats were injected with 5 ug estradiol benzoate, and 44 hours later with saline or naltrexone (3 mg/kg). Rats were sacrificed three hours later. Twenty minutes before decapitation, rats were injected with saline or pargyline (75 mg/kg). Levels of serotonin were measured by HPLC. The areas that were examined were the VMN, ARC-ME, AH, POA, medial nucleus of the amygdala (AME), and the dorsal and lateral midbrain central gray.

* $p < 0.05$.

Results are the mean $\pm$ S.E.M., n = 7 to 8.

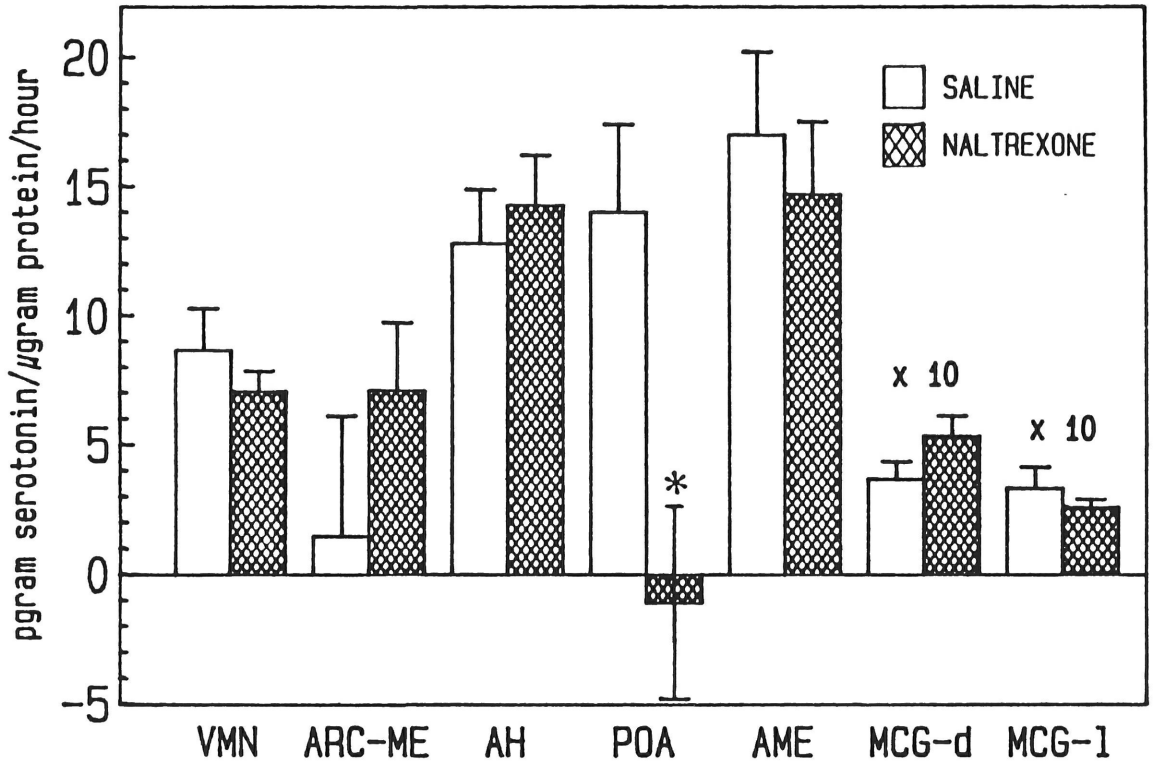


Figure 14. Effects of naltrexone on serotonin turnover in estrogen-primed female rats.

The same rats were used as in Figure 13. The rate of serotonin turnover was estimate from the rate of increase in serotonin levels after treatment with pargyline. The rate of increase was determined by least-squares linear regression.

* $p < 0.05$ compared to saline treatment.

Results are the turnover rate $\pm$ S.E.M. from 13 to 16 determinations.

in the POA of estrogen-primed rats. No change in the accumulation of serotonin after pargyline was noted in the other areas examined.

Discussion

Thus, naltrexone decreases serotonin turnover in the POA, an area of the brain where the expression of lordosis is inhibited by increased serotonergic (Ward, Crowley and Zemlan, 1973; Zemlan, Ward, Crowley and Margules, 1973; Foreman and Moss, 1978a) or opioid activity (Sirinathsinghji, 1986). This action of naltrexone on serotonin turnover occurs at a time when naltrexone would be facilitating sexual behavior. The decrease in serotonin turnover after naltrexone in the POA is consistent with our hypothesis that opiates may be regulating lordosis through actions on serotonergic systems in the POA.

b. Levels of monoamines and metabolites after opiates

Methods

Female rats were ovariectomized, and injected with estradiol benzoate (5 ug s.c.) seven days later. After forty-two hours, rats were subcutaneously injected with saline, naltrexone (3 mg/kg), or morphine (2 mg/kg). This dose of naltrexone will facilitate sexual behavior after three hours, while the morphine will inhibit behavior after one hour. All rats were sacrificed one hour after the last injection. Brain nuclei were removed by the Palkovits

(1973) "punch" technique. Levels of norepinephrine, dopamine, serotonin and 5-hydroxyindole acetic acid in discrete brain nuclei were measured by HPLC with electrochemical detection.

Results

Morphine increased levels of 5-HIAA in the medial preoptic area after one hour compared to saline treated rats (Figure 15). Levels of 5-HIAA in the MCG were decreased by naltrexone. Neither morphine nor naltrexone altered levels of norepinephrine or dopamine after one hour in any of the brain regions that were examined.

Discussion

The increase in 5-HIAA levels in the POA suggests that morphine increased the rate of serotonin turnover. In the MCG, naltrexone decreased serotonin turnover as indicated by a decrease in levels of 5-HIAA. In agreement with previous results, serotonin turnover in the VMN was not altered by treatment with opiates.

c. NSD method

Introduction

The rate of accumulation of 5-HTP has been used as an index of serotonin turnover, though results with this method do not always agree with results using the pargyline method. King and colleagues (1986) reported that progesterone increased the synthesis of 5-hydroxytryptophan in the

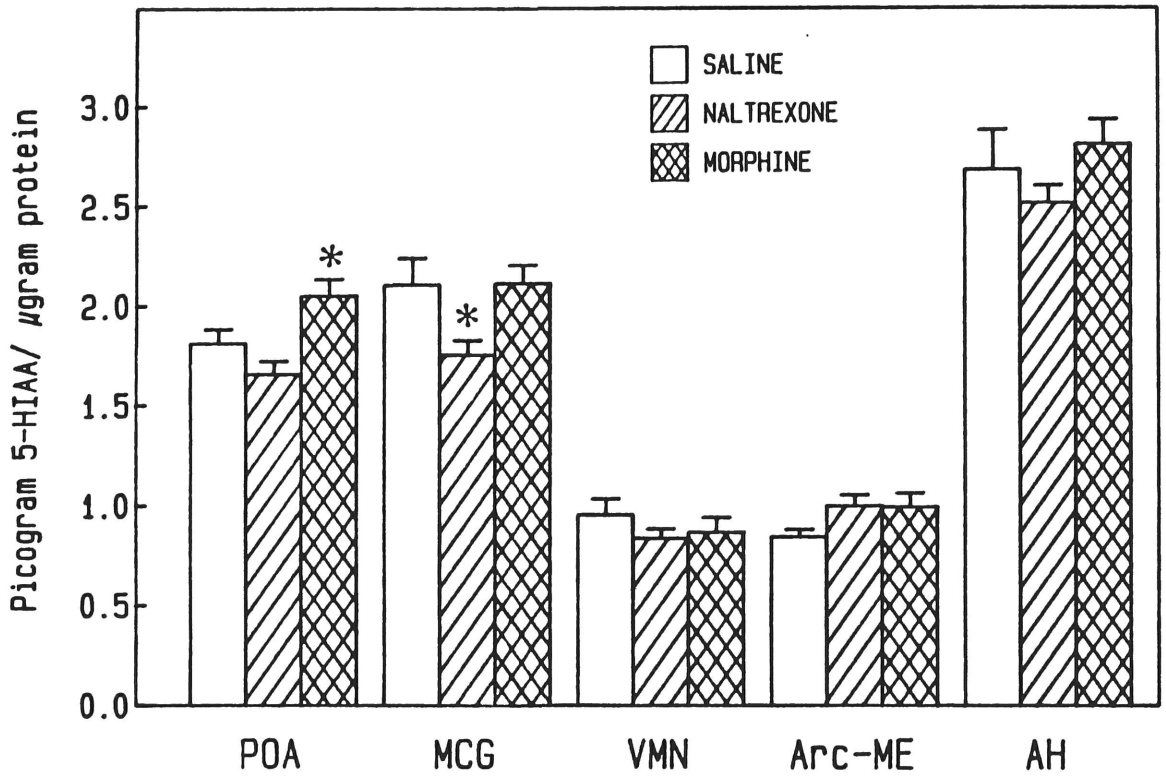


Figure 15. Effects of opiates on 5-hydroxyindoleacetic acid levels in the brain.

Female rats were ovariectomized and seven days later injected with 5 ug estradiol benzoate. After 42 hours, saline, naltrexone hydrochloride (3 mg/kg), or morphine sulfate (2 mg/kg) were injected subcutaneously, and rats were sacrificed one hour later. Levels of monoamines in the medial preoptic area (POA), midbrain central gray (MCG), ventromedial nucleus (VMN), arcuate nucleus-median eminence (ARC-ME), and anterior hypothalamus (AH) were measured by HPLC with electrochemical detection. Values of 5-HIAA are expressed as mean $\pm$ S.E.M., n = 9 or 10.

* $p < 0.05$ compared to saline.

preoptic area-anterior hypothalamus, but there was no effect on 5-HTP synthesis in the medial basal hypothalamus. Other investigators have used the pargyline method to measure serotonin turnover in discrete areas of the hypothalamus-preoptic area. Progesterone increased serotonin turnover in the medial preoptic area (Johnson and Crowley, 1986). However, in the ventromedial nucleus of the hypothalamus, progesterone decreased serotonin turnover (Johnson and Crowley, 1986; Renner, Krey and Luine, 1987).

In these experiments, two different protocols were used. First, naltrexone's and progesterone's effects on serotonin turnover in estrogen primed rats were examined at one hour and at three hours after treatment. In the second set of experiments, morphine's effects on serotonin turnover in estrogen and progesterone treated animals were studied.

Methods

Female rats were ovariectomized and injected with estradiol benzoate (5 ug s.c.) five days later. After forty-two hours, saline, naltrexone (3 mg/kg), or progesterone (500 ug) were injected subcutaneously. Rats were sacrificed one hour later. Neither progesterone nor naltrexone facilitated lordosis after one hour. The aromatic amino acid decarboxylase inhibitor NSD-1015 (100 mg/kg) was injected thirty minutes before decapitation. Levels of 5-hydroxytryptophan in discrete brain nuclei were measured by HPLC with electrochemical detection.

Results

Treatment with NSD-1015 increased levels of 5-HTP in the brain (Figure 16). Neither naltrexone nor progesterone altered the rate of accumulation of 5-HTP in the VMN, POA or AH after one hour.

Discussion

The inability of the treatments to alter the rate of accumulation of 5-HTP suggests that naltrexone and progesterone are not altering the activity of tryptophan hydroxylase one hour after treatment, a time when these treatments do not facilitate lordosis.

Methods

Female rats were ovariectomized and injected with estradiol benzoate (5 ug s.c.) five days later. After forty-two hours, saline, naltrexone (3 mg/kg), or progesterone (500 ug) were injected subcutaneously. Rats were sacrificed three hours later. After three hours, both naltrexone and progesterone facilitate sexual behavior in estrogen primed female rats. The aromatic amino acid decarboxylase inhibitor NSD-1015 (50 mg/kg) was injected thirty minutes before decapitation. Levels of 5-hydroxytryptophan in discrete brain nuclei were measured by HPLC with electrochemical detection.

Results

Both naltrexone and progesterone increased the rate of accumulation of 5-hydroxytryptophan in the medial preoptic

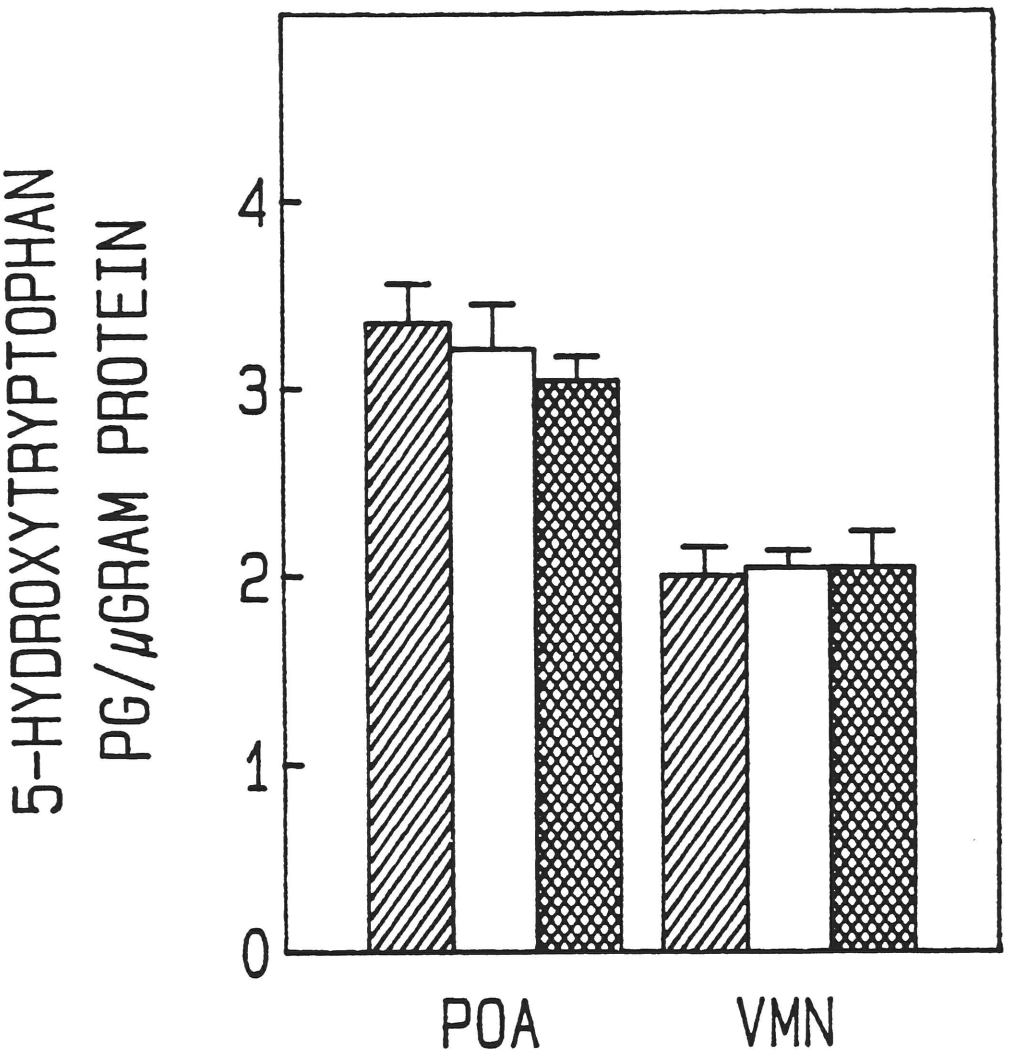


Figure 16. Effect of naltrexone on serotonin synthesis after one hour.

Estrogen-primed (5 ug) female rats were injected with saline (striped bars), naltrexone (3 mg/kg, open bars), or progesterone (500 ug, crosshatched bars), and sacrificed one hour later. Thirty minutes before decapitation, rats were injected with the aromatic amino acid decarboxylase inhibitor NSD-1015. Levels of 5-hydroxytryptophan in the POA and VMN were measured by HPLC with electrochemical detection. Results are the mean $\pm$ S.E.M. for 8 to 12 determinations for each group.

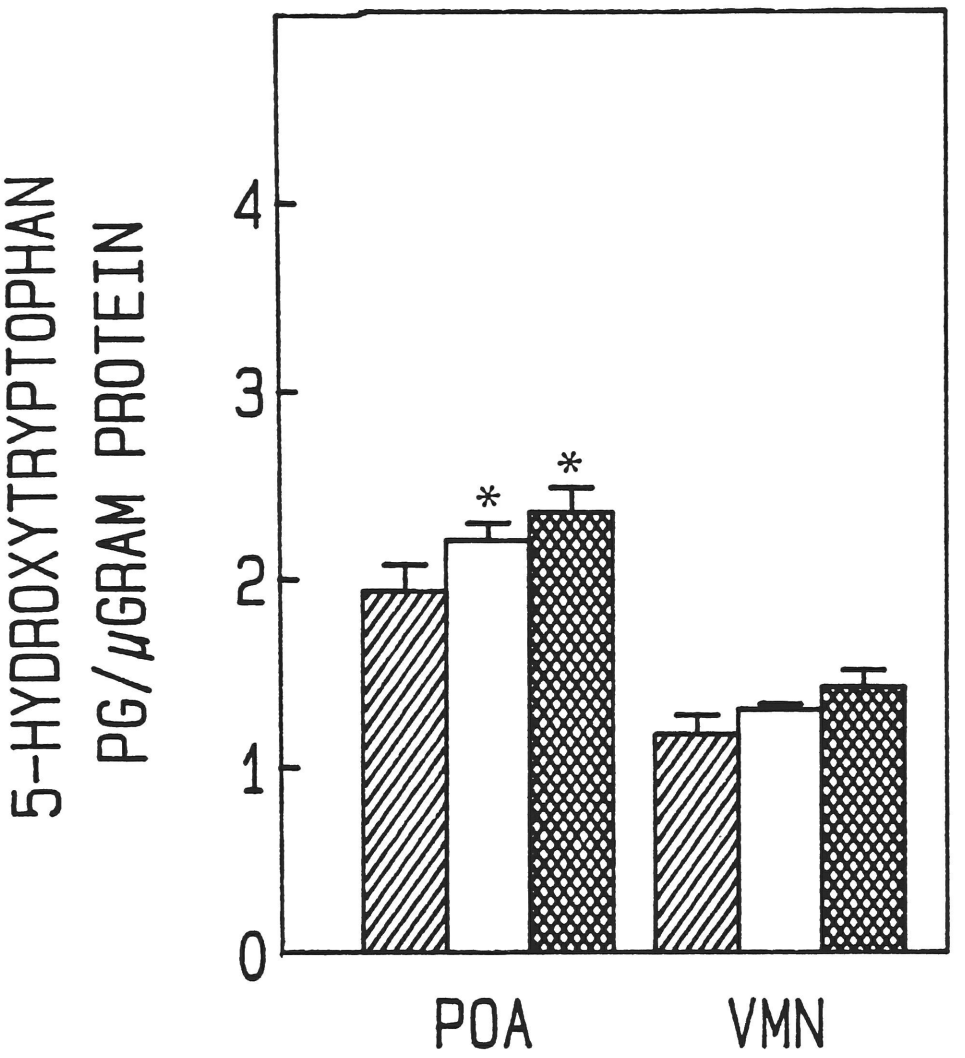


Figure 17. Effect of naltrexone on serotonin synthesis after three hours.

Estrogen-primed female rats (5 ug estradiol benzoate) were injected with saline (striped bars), naltrexone (3 mg/kg, open bars), or progesterone (500 ug, crosshatched bars). Rats were sacrificed three hours later. Thirty minutes before sacrifice, rats were injected with NSD-1015. Levels of 5-hydroxytryptophan were measured in the POA and VMN. Results are the mean $\pm$ S.E.M., n = 9 to 10 for each group.

* $p < 0.05$ compared to saline.

area of estrogen-primed female rats after NSD-1015 (Figure 17). No changes were found in the other brain areas examined.

Discussion

Both naltrexone and progesterone increased the activity of tryptophan hydroxylase in the POA after three hours, when both treatments facilitate lordosis in estrogen-primed female rats. The effects of progesterone are consistent with the results of King and colleagues (1986), who reported that progesterone increased the rate of 5-hydroxytryptophan synthesis in a preoptic area-anterior hypothalamus block, but not in the basomedial hypothalamus. These results suggest that the changes that they report in serotonin synthesis in a POA-AH block occur in the medial preoptic area. Neither naltrexone nor progesterone altered 5-hydroxytryptophan accumulation in the VMN, in agreement with King and collaborators.

Methods

Ovariectomized female rats were injected with estradiol benzoate (5 ug), and forty-four hours later with progesterone (500 ug). Three hours later, rats were injected with morphine sulfate (2 mg/kg) or saline, and sacrificed one hour later. All rats were injected with NSD-1015 (100 mg/kg) thirty minutes before sacrifice. Levels of 5-hydroxytryptophan were measured in the VMN and the POA.

Results

Morphine increased the levels of 5-HTP after NSD-1015 in the POA in estrogen-progesterone primed rats (Figure 18). The accumulation of 5-HTP in the VMN was not altered by morphine

Discussion

The change in 5-HTP accumulation indicates increased activity of tryptophan hydroxylase. This effect of morphine is occurring when morphine would be inhibiting lordosis in estrogen and progesterone treated rats. Morphine did not alter serotonin synthesis in the VMN, which is consistent with the lack of an effect of naltrexone on serotonin turnover in the VMN.

C. CONCLUSIONS

Exogenous opiates alter monoamine turnover in the brain. The changes in turnover are probably not due to changes in levels of the precursor amino acids. Morphine does not change plasma tyrosine or tryptophan levels (Tagliamonte, Tagliamonte, Perez-Cruet, Stern and Gessa, 1971). These changes in monoamine turnover do not occur throughout the brain, but are restricted to discrete areas of the brain. This suggests that the regulation of monoamine turnover occurs at monoaminergic terminals in the projection sites, not at the monoaminergic cell bodies in the brain stem (Koulu, Saavedra, Bjelogrlic, Niwa, Agren and

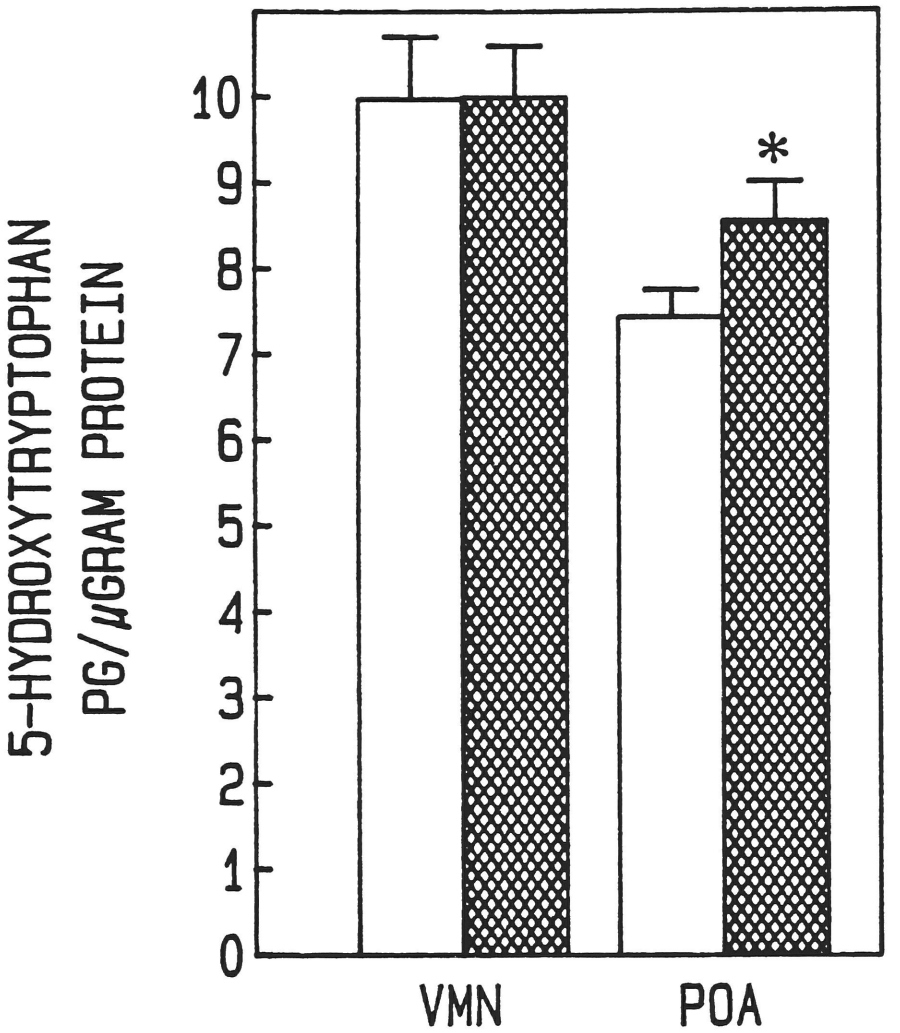


Figure 18. Effect of morphine on serotonin synthesis.

Ovariectomized female rats were injected with estradiol benzoate (5 ug) and progesterone (500 ug 44 hours later). Three hours later rats were injected with morphine sulfate (2 mg/kg) or saline, and sacrificed one hour later. Thirty minutes before sacrifice, rats were injected with NSD-1015 (100 mg/kg). Levels of 5-hydroxytryptophan were measured by HPLC. Saline treatment is the open bars, while the morphine bars are crosshatched.
* $p < 0.05$ compared to saline.
Values are the mean $\pm$ s.e.m. for 9 to 10 determinations.

Linnoila, 1986). In the present experiments, the effects of opiates on monoamine turnover were investigated in areas of the brain important for the regulation of female sexual behavior in the rat. Particular attention was paid to the VMN and POA, since these two areas are extremely important for the regulation of lordosis.

Naltrexone increased norepinephrine turnover in the POA as estimated by the rate of decrease in norepinephrine levels after inhibition of catecholamine synthesis by alpha-methyl-p-tyrosine. Naloxone has been shown to increase norepinephrine release from perifused POA-MBH fragments in vitro (Leadem, Crowley, Simpkins and Kalra, 1985), and opioid peptides inhibit norepinephrine release (Diez-Guerra, Augwood, Emson and Dyer, 1987). The regulation of norepinephrine turnover by opiates in the POA is most likely important for opiate regulation of LHRH secretion (Kalra and Kalra, 1983; 1984). Norepinephrine turnover was not altered in other examined brain regions.

Dopamine levels or turnover were not altered by naltrexone, morphine or progesterone. Lookingland and Moore (1985a) reported that in male rats, morphine decreased dopamine turnover in the median eminence. While this result differs from the results reported here, Lookingland and Moore used male rats, and they examined the median eminence alone. Possible changes in dopamine turnover in the median

eminence of female rats may not be seen in combined arcuate nucleus-median eminence tissue samples.

Exogenous opiates altered indices of serotonin turnover in the POA. Johnson and Crowley (1984) reported opiate regulation of serotonin turnover in the POA in ovariectomized rats or when an opiate antagonist was given at the same time as estrogen treatment. But when using a paradigm similar to that used here, naloxone (10 mg/kg) did not decrease serotonin turnover in the POA (Johnson and Crowley, 1986). The opiate dependent changes in serotonin turnover in the POA reported here are consistent with the behavioral actions of serotonergic drugs in the POA. Morphine increased the rate of accumulation of 5-HTP in estrogen and progesterone primed female rats. Naltrexone decreased the accumulation of serotonin after pargyline in estrogen primed rats. These changes in serotonin turnover occur at times when naltrexone and morphine respectively facilitate or inhibit sexual behavior. Morphine also increased levels of 5-HIAA after one hour in estrogen treated rats. In contrast with the previous results, naltrexone also increased the rate of accumulation of 5-HTP in estrogen-primed rats after three hours, though the effect was small. Thus, at three hours naltrexone decreases the turnover rate of serotonin, but increases the rate of synthesis. This is consistent with the increase in serotonin levels after naltrexone in the "pargyline"

turnover experiment. A similar dissociation between the rates of serotonin turnover and synthesis is seen in the VMN after treatment with progesterone in estrogen primed female rats. Using the pargyline method, two independent groups have reported that progesterone decreases the rate of serotonin accumulation in the VMN (Johnson and Crowley, 1986; Renner, Krey and Luine, 1987), indicating decreased turnover. However, King and colleagues (1986) and the data reported here, show that progesterone does not alter the accumulation of 5-HTP, whether measured in the VMN or the basomedial hypothalamus. This dissociation between the rate of synthesis and turnover may be due to the nature of our experimental treatment. Reproductive behavior in cycling female rats is the product of synchronized changes in hormone and neurotransmitter systems. Treatment with morphine or naltrexone activates or blocks only one of these systems, the endogenous opiates. The effects on serotonergic systems may not be comparable to physiological stimuli, which may be regulating serotonergic neurons at several levels.

In the nigro-neostriatal dopaminergic system, inhibiting the firing rate of dopaminergic neurons decreased the rate of dopamine release, but increased the rate of dopamine synthesis (Roth, Salzman and Nowycky, 1978). The interruption of impulse flow in the dopaminergic neurons, either by pharmacological treatment with gamma-butyrolactone

(Morgenroth, Walters and Roth, 1976; Walters and Roth, 1976) or by lesion of the axonal pathways (Anden, Corrodi, Fuxe and Ungerstedt, 1971; Morgenroth, Walters and Roth, 1976), increases dopamine levels in the striatum and the activity of tyrosine hydroxylase. The increase in tyrosine hydroxylase activity is accompanied by an increase in the affinity of the enzyme for the substrate tyrosine and the pterin cofactor (Morgenroth, Walters and Roth, 1976), and a decrease in the affinity for the end-product inhibitor dopamine (Morgenroth, Walters and Roth, 1976), thereby decreasing the sensitivity of tyrosine hydroxylase to feedback inhibition by increased levels of dopamine (Walters and Roth, 1976). Chelation of endogenous calcium by EGTA increased tyrosine hydroxylase activity, while treatment with dopamine agonists in vivo or the addition of calcium to the incubation medium reversed the increase in tyrosine hydroxylase activity caused by either gamma-butyrolactone or the lesion (Morgenroth, Walters and Roth, 1976). Roth, Salzman and Nowycky (1978) proposed that changes in calcium fluxes in the dopaminergic nerve terminals might be responsible for the impulse blockade-dependent changes in tyrosine hydroxylase activity. Blockade of activity in dopaminergic neurons would prevent the influx of calcium due to depolarization of the nerve terminals and the activation of presynaptic dopamine receptors. The decreased levels of calcium would then activate tyrosine hydroxylase. These

opposite changes in dopamine release and tyrosine hydroxylase activity after decreasing impulse flow in the dopaminergic neurons are similar to the effects of naltrexone on serotonergic activity. Naltrexone decreased serotonin turnover in the preoptic area, but had no effect or slightly increased tryptophan hydroxylase activity.

Treatments with opiates did not alter serotonin turnover in the ventromedial nucleus of the hypothalamus. While hormone dependent changes in serotonin turnover are thought to be important in the control of lordosis (Johnson and Crowley, 1986; Renner, Krey and Luine, 1987), the facilitation of behavior by naltrexone does not require changes in serotonin turnover in the VMN.

VI. REGULATION OF SEROTONIN RECEPTORS BY OPIATES

In addition to examining the regulation of serotonin turnover by opiates, there are other ways to examine opiate regulation of serotonin systems in the brain. One indirect method would be to measure changes in serotonergic activity by measuring levels of serotonergic receptors.

In the cerebral cortex, morphine decreases the release of norepinephrine (Hagan and Hughes, 1984, Werling, Brown and Cox, 1987). Chronic treatment with morphine for three days, which suppresses noradrenergic activity, increases the number of alpha-$_2$ adrenergic receptors in the cortex (Hamburg and Tallman, 1981) and increases the responsiveness of the cyclic AMP system to norepinephrine (Llorens, Martres, Baudry and Schwartz, 1978). Alpha-$_2$ receptors are also increased after lesioning noradrenergic terminals with the selective catecholaminergic neurotoxin 6-hydroxydopamine (U'Prichard, Bechtel, Rouot and Snyder, 1981). Potentiation of the actions of monoamines by chronic treatment with the monoamine oxidase inhibitor clorgyline decreased alpha-$_2$ receptor binding, and systemic infusion of catecholamines decreases renal cortical alpha-$_1$ and beta-adrenergic receptors (Snavely, Ziegler and Insel, 1985).

If opiate agonists increase the rate of serotonin release, chronic treatment with morphine may decrease the number of serotonin receptors. The opposite action would be expected after treatment with opiate antagonists. Levels of

serotonin-1 (5-HT_1) receptors in the brain were examined after two days of continuous treatment with morphine or naltrexone. 5-HT_1 receptors were measured rather than 5-HT_2 receptors, because levels of 5-HT_1 receptors are higher in the hypothalamus (Pazos and Palacios, 1985; Pazos, Cortes and Palacios, 1985), and one subtype, the 5-HT_{1A} receptor, has been implicated in the inhibition of sexual behavior by serotonin (Ahlenius, Fernandez-Guasti, Hjorth and Larsson, 1986; Mendelson and Gorzalka, 1986a, 1986b).

Methods

Ovariectomized female rats were implanted with Alzet minipumps containing morphine sulfate (70 mg/ml saline), naltrexone hydrochloride (70 mg/ml saline) or saline. The rate of infusion of the opiates was approximately 0.35 mg/kg/hr. Rats were sacrificed 48 hours after implantation with the minipumps. This protocol was selected because the identical treatment with morphine produces tolerance to morphine induced bradycardia (Kiang, Dewey and Wei, 1983). Levels of LH in the plasma were measured by Dr. L. Krey using a radioimmunoassay technique (Krey and Parsons, 1982). Levels of 5-HT_1 receptors in the brain were measured using <u>in vitro</u> receptor autoradiography with ^3H-serotonin as the ligand (Figure 19).

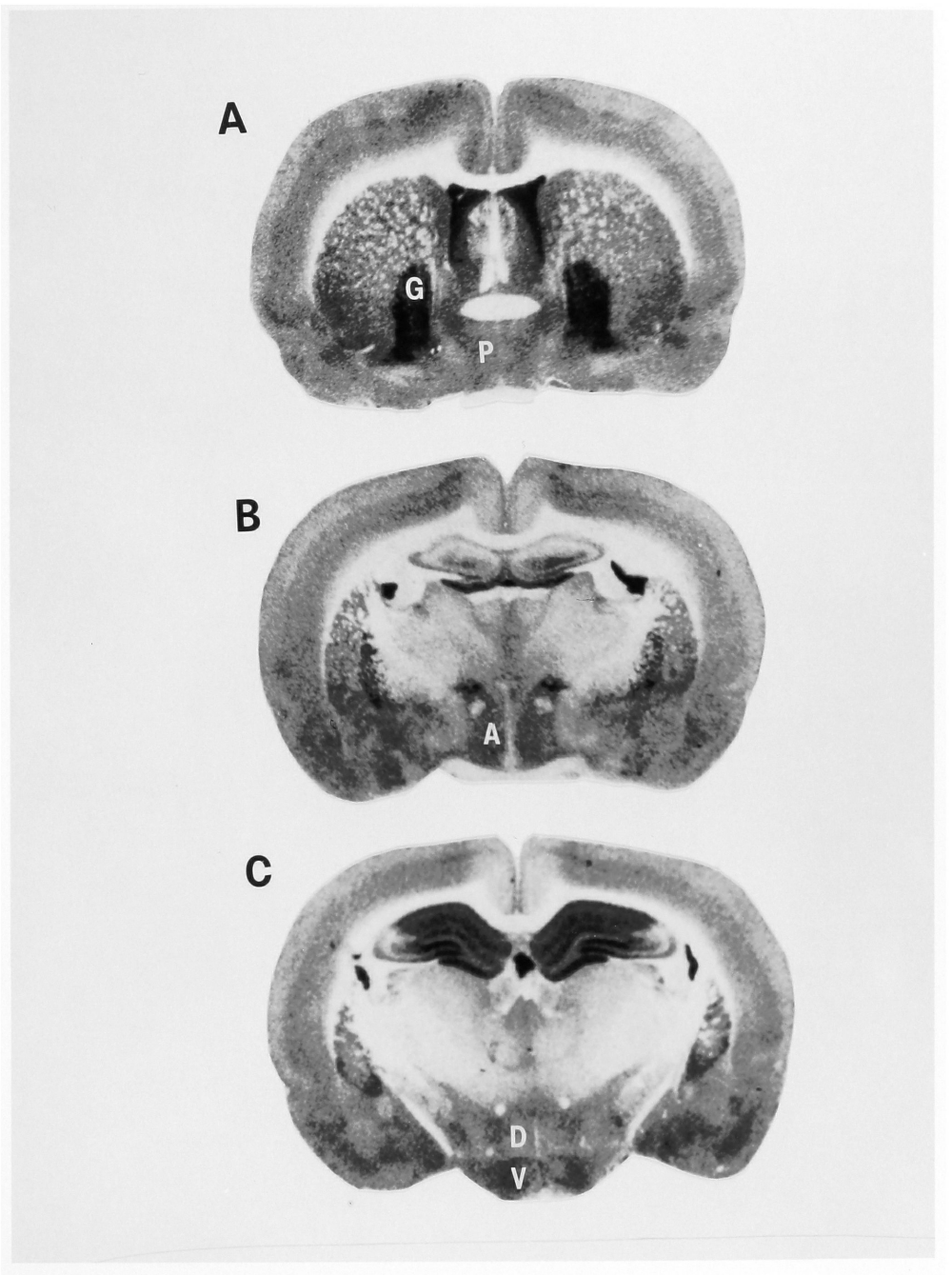
Results

Chronic treatment with morphine or naltrexone did not alter plasma levels of luteinizing hormone (Table 4). In

Figure 19. Binding of ^{3}H-serotonin to serotonin-$_1$ receptors in rat brain.

Binding to serotonin-$_1$ receptors was assessed by incubating brain sections from the level of the preoptic area (A), the anterior hypothalaus (B), or the ventromedial nucleus of the hypothalamus (C) with ^{3}H-serotonin.

Abbreviations: G, globus pallidus; P, medial preoptic area; A, anterior hypothalamus; D, dorsomedial nucleus of the hypothalamus; and V, ventromedial nucleus of the hypothalamus.


A
G
P
B
A
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D
V

Figure 20a-c. Binding of ^{3}H-serotonin to Serotonin-1 receptors in the brain after treatment with opiates.

Ovariectomized female rats were implanted with Alzet minipumps containing saline (open bars), naltrexone·HCl (70 mg/ml, striped bars), or morphine sulfate (70 mg/ml, crosshatched bars). Rats were sacrificed two days later. Binding of Serotonin-1 receptors was assessed by incubating brain sections from the levels of the POA (Figure 20a), the anterior hypothalamus (Figure 20b), and the VMN (Figure 20c) with ^{3}H-serotonin. Levels of binding were analyzed using a DUMAS computer-assisted densitometer. Results are mean $\pm$ S.E.M., n = 5 to 6.

Abbreviations: POA, medial preoptic area; BNST, bed nucleus of the stria terminalis; GP, globus pallidus; VP, ventral pallidum; CP, caudate putamen; PVN, paraventricular nucleus; AH, anterior hypothalamus; ARC, arcuate nucleus; LH, lateral hypothalamus; DMN, dorsomedial nucleus of the hypothalamus; VMN-m, pars medialis of the ventromedial nucleus of the hypothalamus; VMN-l, pars lateralis of the VMN.

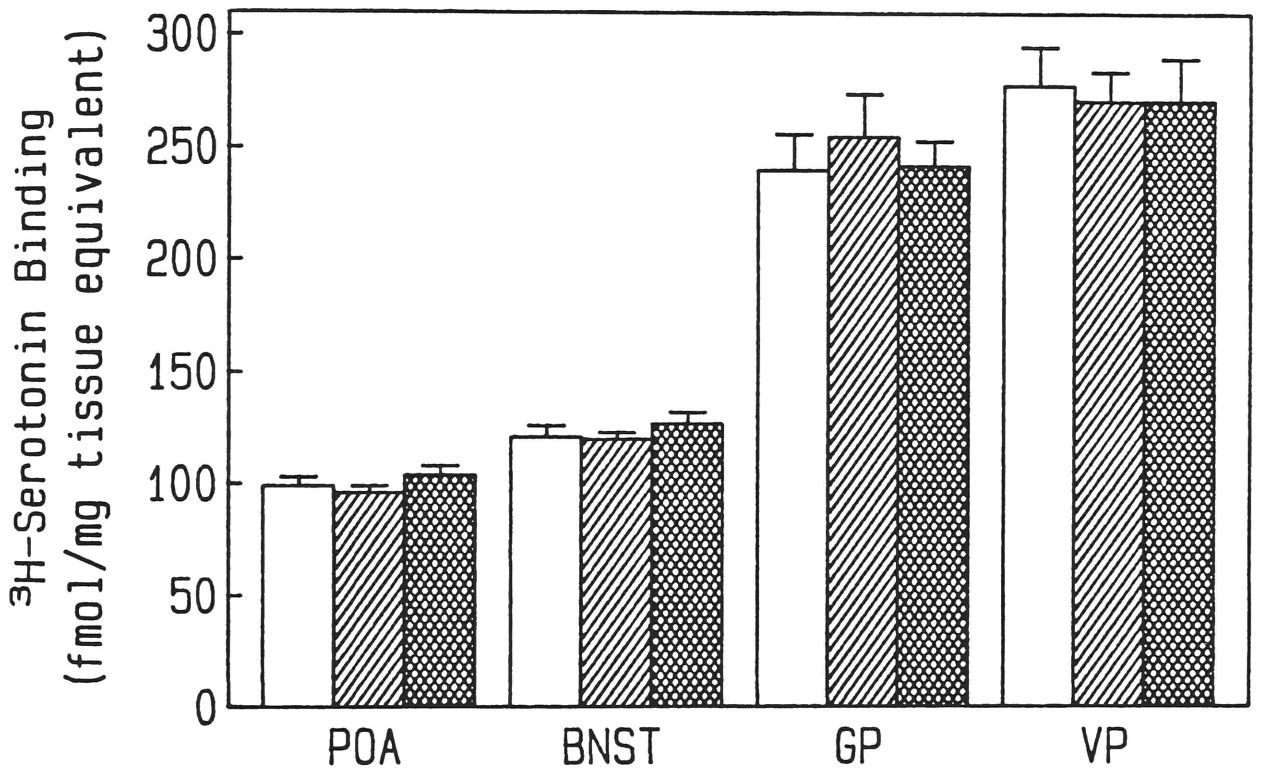

Figure 20a. Binding of ^{3}H-serotonin to serotonin-1 receptors in the brain at the level of the preoptic area.

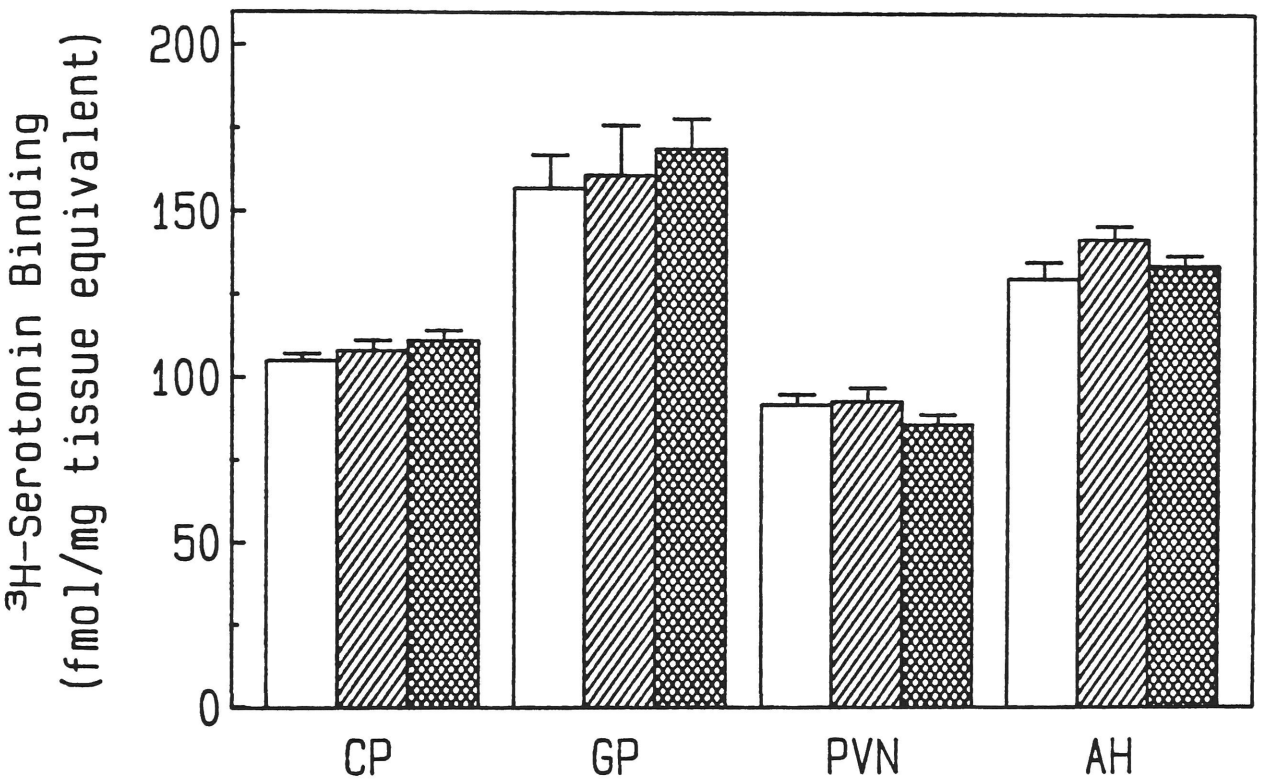

Figure 20b. Binding of ^{3}H-serotonin to Serotonin-1 receptors in the brain at the level of the anterior hypothalamus.

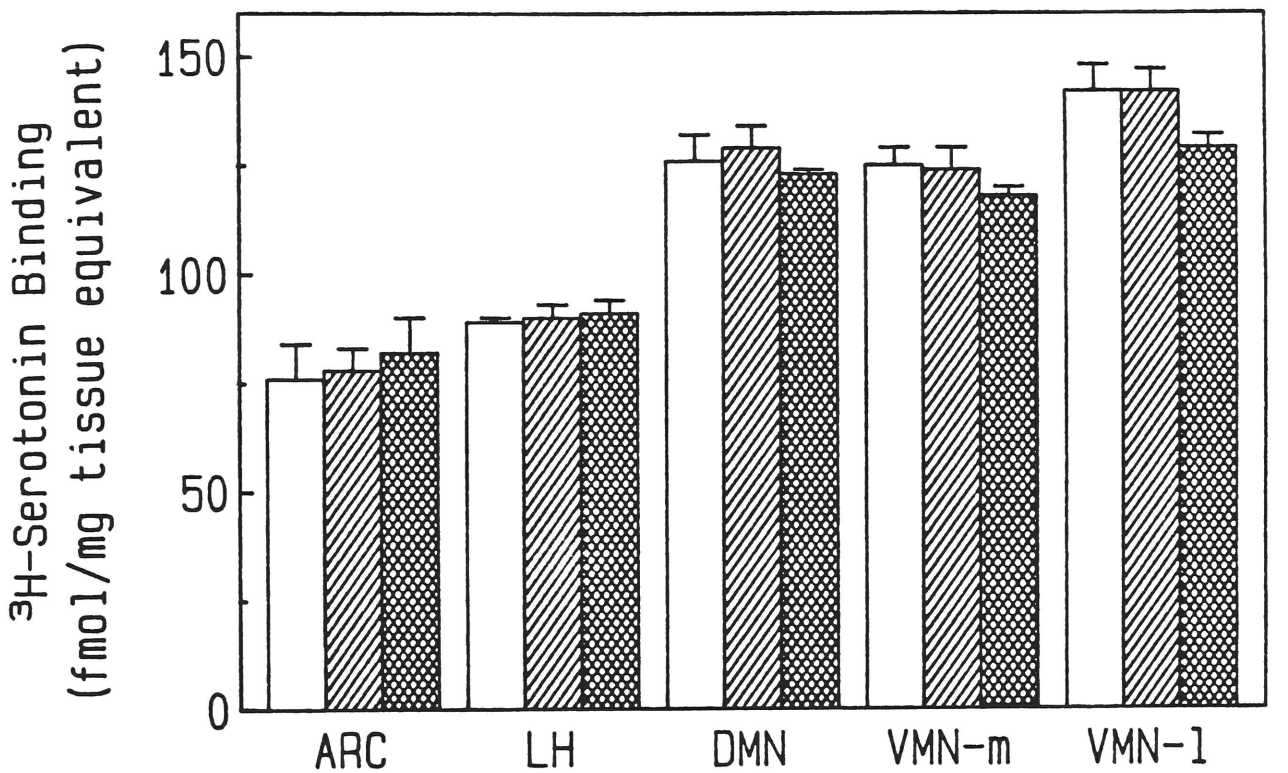

Figure 20c. Binding of ^{3}H-serotonin to Serotonin-1 receptors in the brain at the level of the posterior hypothalamus.

the same rats, this treatment with morphine increased muscarinic acetylcholine receptors in some hippocampal regions, and naltrexone decreased muscarinic receptors in the VMN, the DMN and some regions of the hippocampus (Johnson, Coirini, and McEwen, personal communication). Levels of 5-HT_1 receptors were measured in coronal sections at the level of the POA (Figure 20a), anterior hypothalamus (Figure 20b), and the VMN (Figure 20c). Treatment with opiates did not change ^3H-serotonin binding in any of the brain areas that were examined.

Discussion

While acute treatments with opiates alter LH secretion, treatment for two days with morphine or naltrexone did not alter LH levels. A similar lack of an effect of chronic exposure to naloxone on LH levels has been previously reported (Gabriel and Simpkins, 1983).

After two days of exposure to opiates, the neuroendocrine system, at least with regards to LH secretion, has adjusted to the continual exposure to opiates. This experiment examined if these adaptive changes are occurring at the level of serotonin receptors in response to presumed opiate-induced changes in serotonin turnover. Serotonin-1 receptors were not altered by the opiate treatments. The treatment may not have been of sufficient duration to produce measurable changes in serotonin binding. Muscarinic receptors were altered by the

treatments, indicating that opiate function was changed by the treatments, though muscarinic receptors may be more sensitive to regulation by opiates than serotonin receptors. Another possibility is the sensitivity of the method for measuring serotonin receptors. Serotonin-1 receptors have been divided into three subtypes, the 1A, 1B AND 1C subtypes (Peroutka, 1986), and ^{3}H-serotonin binds to all three subtypes. Both 5-HT_{1A} and 5-HT_{1B} receptors are present in the hypothalamus (Pazos and Palacios, 1985). If the changes are occurring in only one subtype of serotonin receptor, the change may not be reflected in changes in total 5-HT_1 receptors. The lack of an effect of the opiate treatments on serotonin receptors suggests that the adaptive changes may be occurring at a site(s) other than the serotonin receptors. Chronic morphine did not alter serotonin levels or turnover, or change the activity of tryptophan hydroxylase (Schechter, Lovenberg and Sjoerdsma, 1972). The adaptation in the system may involve changes in opiate receptors, since chronic treatment with opiate antagonists has been shown to upregulate numbers of opiate receptors (Tempel, Gardner, and Zukin, 1984; Tempel, Crain, Peterson, Simon and Zukin, 1986; Zukin and Tempel, 1986).

VII. OPIATE RECEPTORS AFTER SEROTONERGIC LESIONS

Interactions between serotonergic and opiate systems were found at the level of behavior and at the level of neurotransmitter turnover. An intact serotonergic system is required for the behavioral actions of opiates. Morphine and naltrexone regulate serotonin turnover in the POA, an area of the brain important for opiate mediation of lordosis. Opiates may be acting at presynaptic opiate receptors on serotonergic terminals in the POA to regulate serotonin release (Figure 21). This has been found to be the case in the striatum (Parenti, Tirone, Olgiati and Groppetti, 1983), where morphine increases serotonin turnover. To test this hypothesis, levels of opiate receptors were examined after lesions of serotonergic terminals in the brain, which would result in the loss of presynaptic receptors on the serotonergic terminals.

Methods

Serotonergic terminals in the brain were lesioned by intraventricular infusion of the selective serotonergic neurotoxin 5,7-dihydroxytryptamine. A similar treatment was found to reduce serotonin levels in the hypothalamus and preoptic area by at least 90% (Fischette, Nock and Renner, 1987). Control rats were untreated. One week after the lesion, the rats were sacrificed and their brains processed for <u>in vitro</u> receptor autoradiography for mu and delta opiate receptors (Tempel and Zukin, 1987). Mu opiate

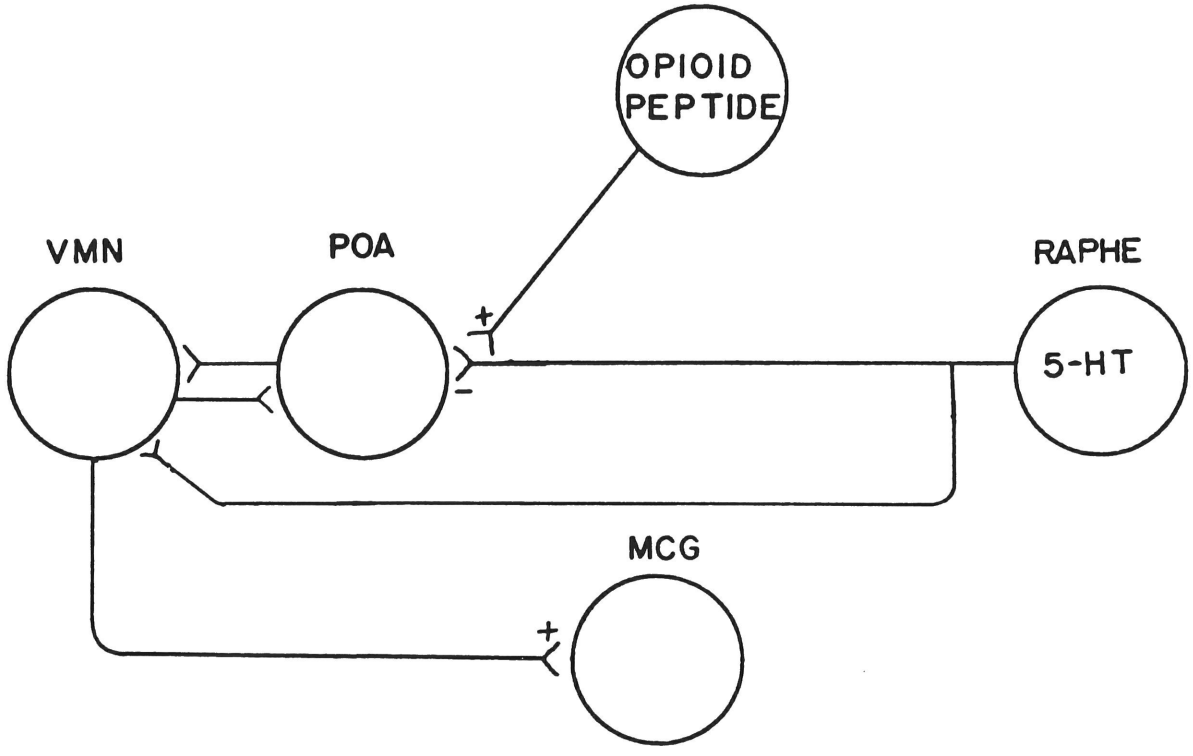

Figure 21. Model for opiate regulation of lordosis

receptors were labeled with ^{3}H-[D-Ala^{2},N-MePhe^{4}, Gly-ol^{5}]Enkephalin (DAGO). For delta receptor binding, brain sections were incubated with ^{3}H-[D-Pen^{2}, D-Pen^{5}]Enkephalin (DPDPE). Opiate receptors were measured in the POA, MCG, ventrolateral and dorsomedial portions of the VMN and the DMN.

Results

Treatment with 5,7-DHT decreased levels of serotonin in the brain by at least 80%, but did not alter levels of norepinephrine or dopamine. Levels of mu and delta opiate receptors were measured in coronal sections at the level of the POA (Figure 22a,d), the VMN (Figure 22b,e), and the MCG (Figure 22 c,f). The serotonergic lesion decreased ^{3}H-DAGO binding in the POA by 28% and in the MCG by 23% (Figure 23). Binding of ^{3}H-DPDPE to delta receptors was decreased in the POA by 27%, and in the DMN by 19% (Figure 24). Neither ^{3}H-DAGO binding nor ^{3}H-DPDPE binding was decreased in eithe

the ventrolateral or the dorsomedial portion of the VMN.

Discussion

The decrease in binding of ^{3}H-DAGO and ^{3}H-DPDPE to opiate receptors in the POA, DMN and MCG after serotonergic lesions provides additional evidence for the existence of interactions between opiate and serotonergic systems in these areas. Opiates altered serotonin turnover in the POA and MCG, two areas where the lesion decreased opiate binding. The inability of opiates to regulate serotonin

Table 4. Plasma LH levels after two days of opiate treatment.

	LH levels
Saline	277 ± 68
Naltrexone	441 ± 69
Morphine	284 ± 81

Ovariectomized female rats were implanted with Alzet minipumps containing saline, naltrexone hydrochloride (70 mg/ml), or morphine sulfate (70 mg/ml). Rats were sacrificed two days later, and trunk blood was collected for radioimmunoassay of LH (Krey and Parsons, 1982).

Values for LH levels are ng NIAMDD-RP-1/ml (mean ± S.E.M.) for 6 rats.

Figure 22a-f. Opiate receptors in the brain after serotonergic lesions.

Serotonergic terminals in the brain were lesioned by intraventricular infusion of 5,7-dihydroxytryptamine. Binding to mu opiate receptors was assessed by incubating brain sections with ^{3}H-DAGO, and delta receptor binding was measured using ^{3}H-DPDPE. Brain sections were taken from the level of the preoptic area, the midbrain central gray, and the ventromedial nucleus.

Abbreviations: P, medial preoptic area; D, dorsomedial nucleus of the hypothalamus; V, ventromedial nucleus of the hypothalamus, and M, midbrain central gray.

22a. Levels of mu opiate receptors at the level of the POA.

22b. Levels of mu opiate receptors at the level of the VMN.

22c. Levels of mu opiate receptors at the level of the MCG.

22d. Levels of delta opiate receptors at the level of the POA.

22e. Levels of delta opiate receptors at the level of the VMN.

22f. Levels of delta opiate receptors at the level of the MCG.

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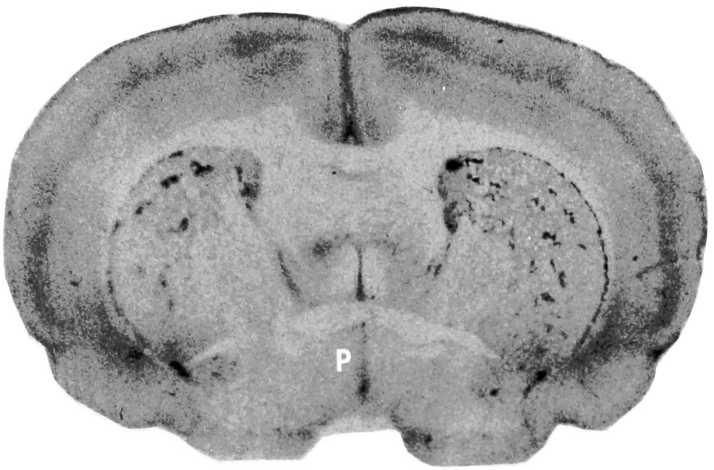

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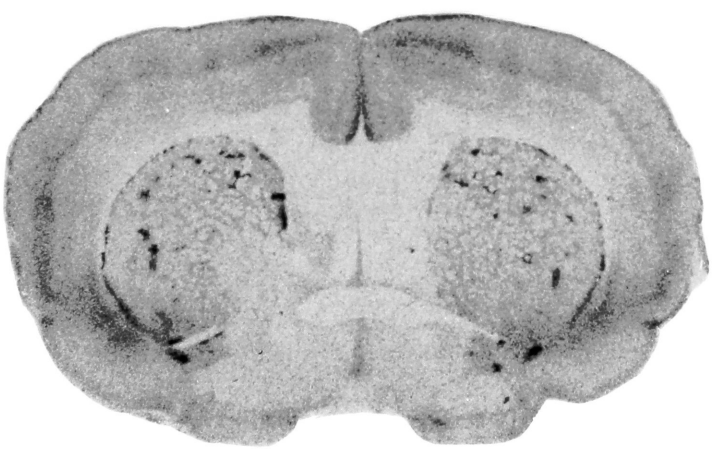

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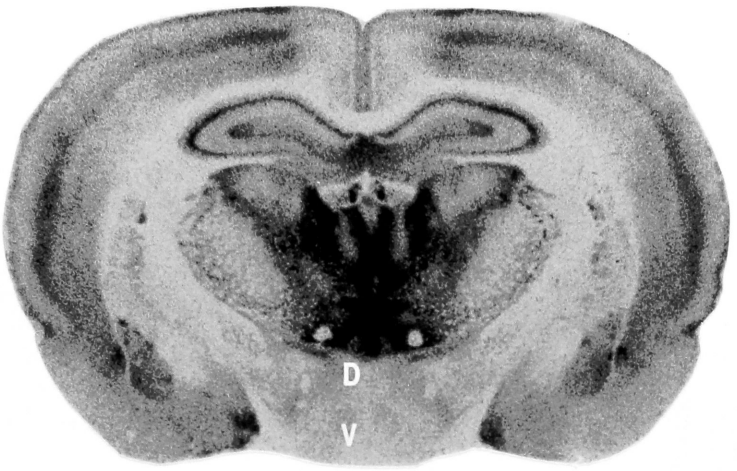

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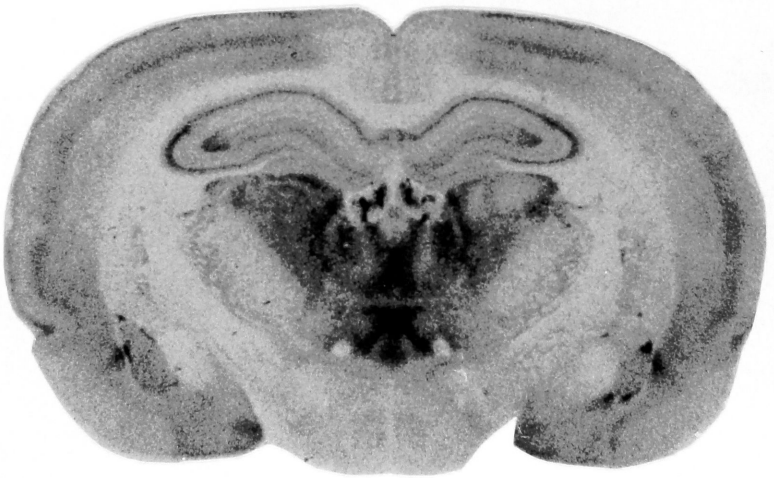

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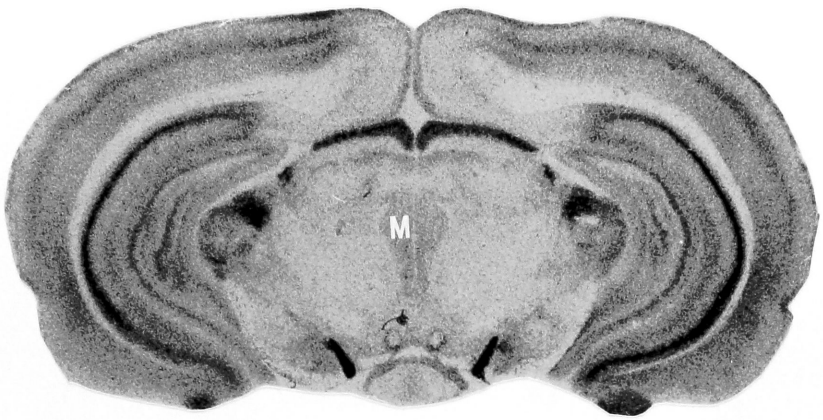

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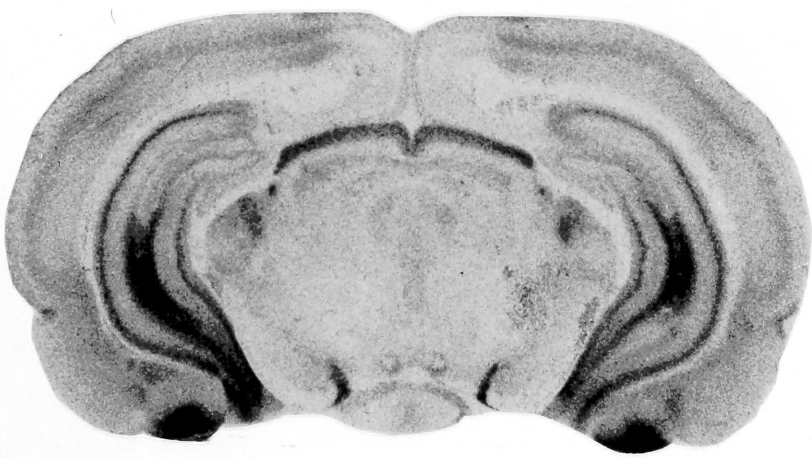

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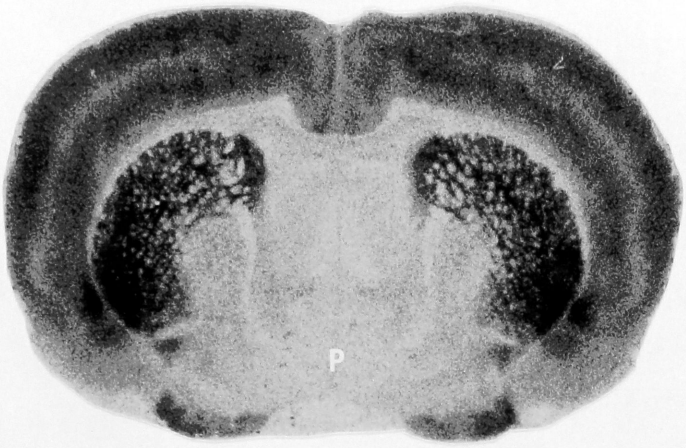

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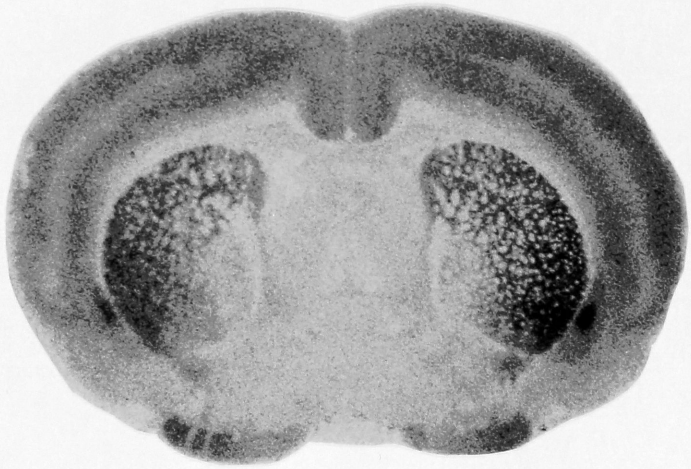

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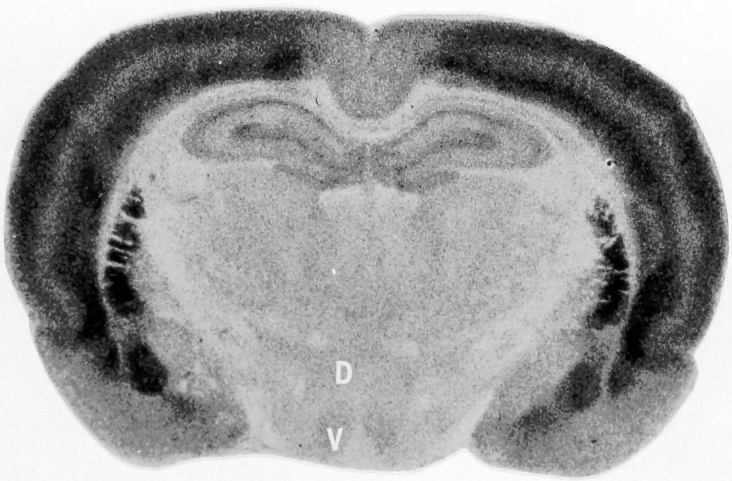

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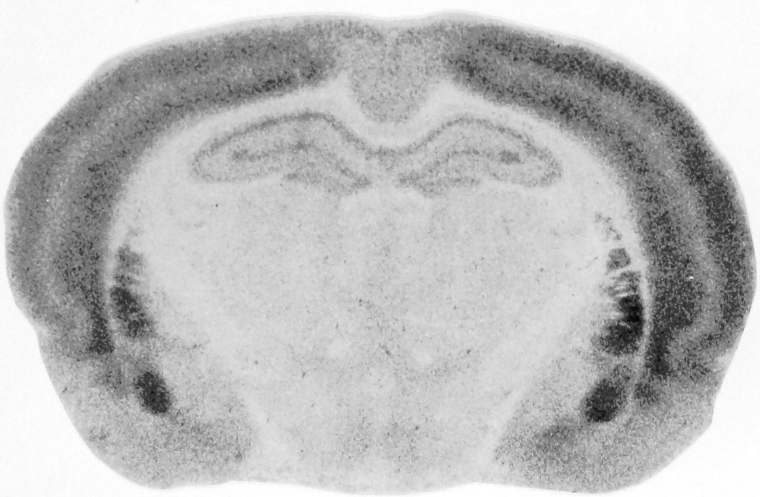

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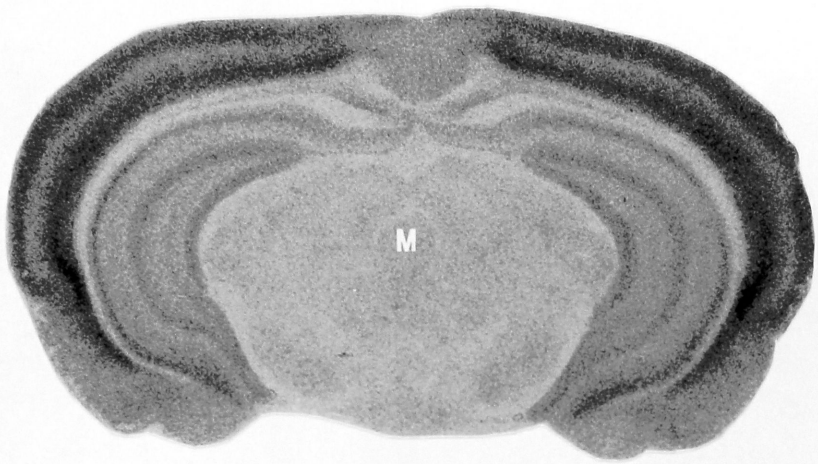

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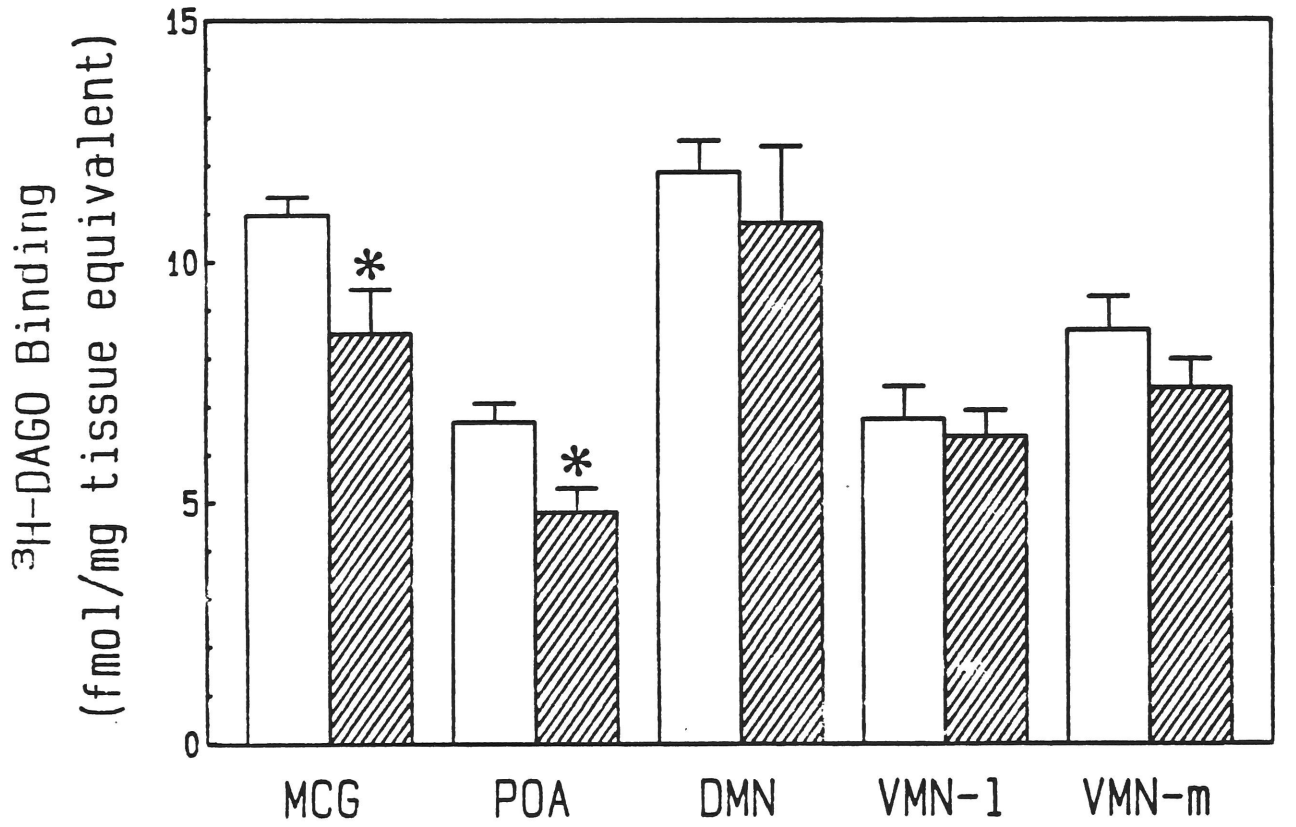

Figure 23. Binding of ^{3}H-DAGO to mu opiate receptors in the brain of untreated rats (open bars), and after serotonergic lesions (striped bars). Results are mean $\pm$ S.E.M., n = 4-7. See Figure 20 for abbreviations.
* $p < 0.05$

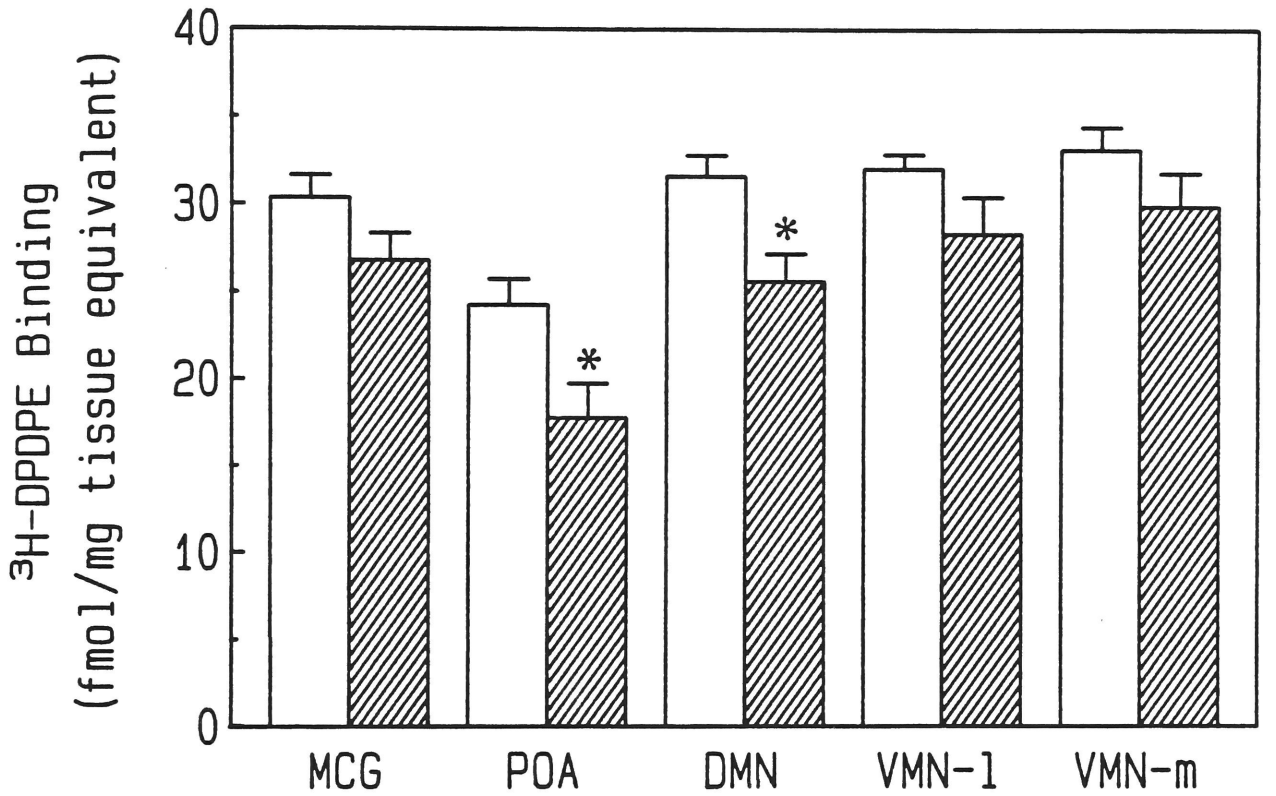

Figure 24. Binding of ^{3}H-DPDPE to delta opiate receptors in the brains of untreated rats (open bars) and rats with serotonergic lesions (striped bars). Results are mean $\pm$ S.E.M., n = 6-7.

See Figure 20 for abbreviations.

* $p < 0.05$

turnover in the ventrolateral VMN was correlated with the lack of a decrease in opiate binding after the serotonergic lesion. Thus, opiate regulation of serotonin turnover can be correlated with decreases in opiate binding after serotonergic lesions.

The decrease in opiate binding in some areas after the serotonergic lesion suggests that a subpopulation of the opiate receptors in these areas are located on serotonergic terminals. Lesions of terminals results in the loss of these receptors. The decrease in ^{3}H-DAGO binding in the POA and the MCG suggests that 20 to 30% of the mu opiate receptors in these regions are present on serotonergic terminals. A subpopulation of delta opiate receptors in the POA and DMN also appear to be localized on serotonergic terminals, as indicated by the decrease in ^{3}H-DPDPE binding after the lesion. The presence of mu and delta opiate receptors on serotonergic terminals in the POA would provide an anatomical basis for opiate induced changes in serotonin turnover. In the DMN, the presence of delta receptors on serotonergic terminals was not correlated with opiate regulation of serotonin turnover. The DMN has an important role in ingestive behavior, and may be involved in opiate-related feeding (Bernardis and Bellinger, 1987). Serotonergic cell bodies are located in the DMN (Frankfurt, Lauder and Azmitia, 1981), and it appears that it would be worthwhile to further study the role of serotonin in opiate

actions. A portion of mu receptors in the MCG was localized to serotonergic terminals. The role of these receptors in opiate regulation of serotonin turnover is unclear, since opiates altered serotonin turnover in only one experiment.

Further studies are required to determine if the changes in opiate binding are due to changes in number of receptors, or to changes in the affinity of the receptor for the ligand. In the ventrolateral VMN, the absence of a decrease in mu or delta receptors after the serotonergic lesion correlates with the inability of opiates to regulate serotonin turnover in this area. Thus, these data provide a neuroanatomical basis for opiate regulation of serotonin turnover in the POA and the MCG, and the lack of regulation in the VMN. Opiates may be able to act directly on serotonergic endings, and not require intervening connections or neurons.

VIII. CONCLUSIONS

A. OPIATE REGULATION OF LORDOSIS

Opiates are potent inhibitors of gonadotropin secretion in the rat (Bruni, Van Vugt, Marshall and Meites, 1977; Meites, Bruni, Van Vugt and Smith, 1979; Bicknell, 1985). Agonists decrease levels of luteinizing hormone. In addition, opiates inhibit the expression of sexual behavior (Hetta, 1976; Ostrowski, Stapleton, Noble and Reid, 1979; Wiesner and Moss, 1984). While opiate antagonists increase luteinizing hormone levels, many investigators have reported that systemic or intraventricular injection of opiate antagonists did not facilitate sexual behavior (Ostrowski, Noble and Reid, 1981; Wiesner and Moss, 1984; Pfaus and Gorzalka, 1987a). However, many of these investigations examined rapid effects of the opiate antagonists on behavior. The purpose of the present experiments was to further investigate opiate regulation of behavior.

The opiate antagonist naltrexone facilitated sexual behavior in estrogen primed female rats. Only one dose of naltrexone, 3 mg/kg, was able to consistently facilitate behavior. Lordosis quotients were increased from three to four hours after the administration of the opiate antagonist. Naltrexone did not facilitate behavior in rats without estrogen priming. Thus, it appears that endogenous opioid peptides tonically inhibit lordosis. Antagonism of the action of the opiates with naltrexone blocks the opiate

inhibition and allows for the expression of sexual behavior. The action of naltrexone was not due to the release of adrenal steroids nor did it appear to require protein synthesis. The role of specific areas of the brain in opiate regulation of lordosis was also examined. Morphine, administered into either the medial preoptic area or the ventromedial nucleus of the hypothalamus, inhibited sexual behavior. Naltrexone infusion into the POA facilitated lordosis, in agreement with Sirinathsinghi (1986) who reported that naloxone increased lordosis quotients.

Endogenous opioid peptides may be important mediators of the actions of gonadal steroids. Neurons in the medial basal hypothalamus have been reported to contain beta-endorphin, enkephalin, or dynorphin (McGinty and Bloom, 1983; Morrell, McGinty and Pfaff, 1985). A proportion of opioid peptide immunoreactive neurons also accumulate estradiol (Jirikowski, Merchenthaler, Rieger and Stumpf, 1986; Morrell, McGinty and Pfaff, 1985; Romano, Harlan, Shivers, Howells and Pfaff, 1986). Chronic treatment with estradiol results in changes in opiate systems in the brain. Estradiol increased naloxone binding sites (Wilkinson, Bhanot, Wilkonson, and Brawer, 1983) and decreased levels of beta-endorphin (Forman, Marquis and Stevens, 1985; Wardlaw 1982, 1985) and pro-opiomelanocortin mRNA (Wilcox and Roberts, 1985) in the hypothalamus. Opposite actions are seen in enkephalinergic systems after estrogen treatment.

In the POA, ovariectomy decreased immunoreactive enkephalin (Watson, Hoffman Wiegand, 1986), and estrogen increased levels of enkephalin in the POA and MCG (Dupont, Barden, Cusan, Merand, Labrie and Vaudry, 1980). Estrogen increased levels of preproenkephalin in the medial basal hypothalamus (Romano, et al., 1986). Furthermore, endogenous opioid peptides have been implicated in steroid regulation of the LH surge. Opiate peptides tonically inhibit LH secretion (Gabriel, Simpkins and Kalra, 1983), and restrain the initiation of the LH surge (Allen and Kalra, 1986; Lustig, Pfaff and Fishman, 1988). On proestrus in cycling female rats or after progesterone administration, there is a decrease in opioid tone in the hypothalamus-preoptic area (Gabriel, Simpkins and Kalra, 1983; Allen and Kalra, 1986; Petraglia, Locatelli, Facchinetti, Bergamaschi, Genazzani and Cocchi, 1986; Lustig, Pfaff and Fishman, 1988) that allows the neural events to occur which culminate in the preovulatory LH surge. These changes in opioid tone in the hypothalamus, that are important for the regulation of gonadotropin secretion, are occurring at the same time as the facilitation of lordosis. The steroid-dependent decrease in opioid tone at this time may also be important for the coordinated expression of lordosis.

Of particular interest was the investigation of possible substrates for opiate regulation of behavior. The two most likely systems by which opiates would regulate

lordosis are the monoamines and luteinizing hormone releasing hormone (LHRH). LHRH facilitates sexual behavior in estrogen primed rats, and is active after systemic injection (Moss and McCann, 1973; Pfaff, 1973), or infusion into the preoptic area (Moss and Foreman, 1976; Foreman and Moss, 1977; Sirinathsinghji, 1986), the basomedial hypothalamus (Moss and Foreman, 1976; Foreman and Moss, 1977) or the midbrain central gray (Riskind and Moss, 1979; Sakuma and Pfaff, 1980; Sirinathsinghji, 1984). Opiate antagonists increase plasma levels of luteinizing hormone, presumably by increasing secretion of LHRH (Lustig, Pfaff and Fishman, 1988). The facilitation of behavior after intracranial infusion of naloxone into the POA and the MCG are blocked by an LHRH antagonist (Sirinathsinghji, 1984; 1986), and the inhibition of behavior by infusion of beta-endorphin into these same areas is reversed by infusion of LHRH.

Less well studied than opiate-LHRH interactions is the role of opiate interactions with monoaminergic neurotransmitters in the control of lordosis. Monoaminergic systems, particularly serotonergic systems, are known to be important regulators of lordosis (Meyerson, 1964a, 1964b, 1964c). Infusion of serotonin into the hypothalamus-preoptic area inhibits behavior (Foreman and Moss, 1978a), while serotonergic antagonists or lesions of serotonergic terminals facilitate lordotic responding (Ward,

Crowley, Zemlan and Margules, 1975; Foreman and Moss, 1978a; Frankfurt, Renner, Azmitia and Luine, 1985). Serotonergic regulation of lordosis does not appear to involve changes in LHRH release, since the inhibition of behavior after infusion of serotonin agonists into the preoptic area in not reversed by the infusion of LHRH (Foreman and Moss, 1978a). A possible role of serotonergic systems in the opiate regulation of lordosis was suggested by the actions of opiates on serotonin turnover in the brain. Morphine increased serotonin turnover in the POA and the arcuate nucleus, and the effect was reversed by naloxone (Johnston and Moore, 1983). However, in most cases opiate-serotonin interactions have been studied with regard to luteinizing hormone secretion, not the regulation of sexual behavior.

B. OPIATE-SEROTONIN INTERACTIONS IN THE REGULATION OF SEXUAL BEHAVIOR

The role of monoamines in opiate regulation of sexual behavior was investigated. The ability of naltrexone to facilitate lordosis was tested in rats treated with pargyline. In addition, morphine inhibition of lordosis was examined in rats with hypothalamic serotonergic lesions.

Pargyline inhibits monoamine oxidase, the primary degradative enzyme for serotonin and the catecholamines (Luine and Paden, 1982). By blocking their inactivation, pargyline potentiates the action of endogenous monoamines.

Pretreatment with pargyline blocks the facilitation of lordosis by progesterone in estrogen primed rats (Luine and Paden, 1982). Pargyline pretreatment also inhibits naltrexone facilitated behavior, suggesting that inhibitory actions of monoamines are able to antagonize the facilitation of behavior by naltrexone.

Frankfurt, Renner, Azmitia and Luine (1985) reported that hypothalamic serotonergic lesions produce high levels of lordosis after low levels of estrogen, and that this facilitation of behavior lasts for several weeks. In preliminary work, Moreines and McEwen reported that naltrexone was unable to facilitate sexual behavior in rats with hypothalamic serotonergic lesions. The inhibition of behavior by morphine was examined in rats with serotonergic lesions. Morphine, at a dose that inhibits estrogen-progesterone facilitated behavior, was unable to inhibit lordosis in lesioned rats primed with estrogen alone. This suggests that opiates regulate sexual behavior by decreasing serotonin release in the hypothalamus, which then directly inhibit the expression of lordosis.

C. OPIATE REGULATION OF MONOAMINERGIC FUNCTION

Interactions between serotonin and opiates were then examined in the brain. Opiate regulation of serotonin turnover has not been studied with the same paradigms used to study opiate regulation of sexual behavior. Monoamine

turnover and levels of serotonin receptors were examined after treatment with opiates. Levels of opiate receptors were also examined after serotonergic lesions.

a. Opiate regulation of monoamine turnover

In the medial preoptic area, opiates regulated norepinephrine and serotonin turnover. Sirinathsinjghi (1986) and the work presented here provide evidence that the POA is an important site for the behavioral actions of opiates. Naltrexone increased norepinephrine turnover, but decreased the turnover of serotonin. Morphine increased the rate of serotonin turnover. While the role of norepinephrine in the regulation of lordosis is unclear (Caldwell and Clemens, 1986; Davis and Kohl, 1977; Foreman and Moss, 1978; Fernandez-Guasti, Larsson and Beyer, 1985a; 1985b), serotonin acts to inhibit the expression of sexual behavior. Thus, the opiate induced changes in serotonin turnover in the POA are consistent with the behavioral actions of opiates. Opiate agonists, which inhibit behavior, also increase serotonin turnover in the POA, which would also be expected to inhibit behavior. The opposite actions are seen with opiate antagonists. These data provide a neurochemical and anatomical basis for serotonin mediated actions of opiates on sexual behavior.

b. Opiate regulation of serotonin receptors

Serotonin-1 receptors in the brain were not altered by two days of treatment with morphine or naltrexone. The inability of opiates to alter levels of serotonin receptors in areas of the brain where opiates regulate serotonin may be due to: 1) the differential regulation of serotonin-2 receptors or the different subtypes of serotonin-1 receptors or 2) adaptation to the chronic activation or blockade of opiate receptors at a level other than the serotonin receptors.

Serotonin receptors have been divided into two main classes of receptors, serotonin-2 receptors (Pazos, Cortes and Palacios, 1985) and serotonin-1 receptors (Pazos and Palacios, 1985), which have been further divided into three subtypes, the 1A, 1B, and 1C receptors. All three receptor subtypes are labeled by ^{3}H-serotonin. Specific ligands have been developed which preferentially label each type or subtype of receptor. Opiate treatments may alter only serotonin-2 receptors or one of the subtypes of serotonin-1 receptors. Regulation of the different serotonin receptors by opiates can be examined by measuring binding using ligands specific for the different receptors.

Adaptation to the chronic opiate treatments may not have occurred at the level of serotonin receptors. Tolerance develops after long-term exposure to opiate agonists. Chronic treatment with opiate antagonists

increases opiate receptors (Tempel, Gardner, and Zukin, 1984; Tempel, Crain, Peterson, Simon and Zukin, 1986).

c. Opiate receptors after serotonergic lesions

Serotonergic lesions decreased opiate binding in the POA, DMN and MCG. The decreased binding is presumably due to the loss of opiate receptors on serotonergic terminals in these areas. Further studies are required to determine if the decrease in binding is the result of a decrease in the number of binding sites, or a change in the affinity of the binding sites for the ligands. Alternatively, the change in opiate binding could have been the result of decreased serotonergic activity. To exclude this possibility, opiate receptors could be measured after inhibiting serotonin synthesis with p-chlorophenylalanine, an inhibitor of tryptophan hydroxylase. p-Chlorophenylalanine, p-CPA, would decrease serotonin activity, but would not lesion serotonergic terminals. If the decrease in opiate binding was due to the loss of serotonergic terminals, p-CPA would not be expected to decrease opiate binding (Parenti, Tirone, Olgiati and Groppetti, 1983).

D. ROLE OF THE PREOPTIC AREA IN THE OPIATE REGULATION OF LORDOSIS

The preoptic area is an important site for opiate regulation of reproductive functions. Parameters of opiate systems in the preoptic area are sexually dimorphic (Hammer,

1984; Watson, Hoffman and Wiegand, 1986). In the female, opiate systems vary with the stage of the estrous cycle, and are modulated by exogenous gonadal steroids in ovariectomized rats (Hammer, 1984; Watson, Hoffman and Wiegand, 1986). The enkephalinergic fiber system of the periventricular region of the preoptic area is denser in females compared to males (Watson, Hoffman and Wiegand, 1986). The greater density in females is estrogen dependent. In cycling female rats, the density of enkephalin immunoreactive fibers is highest on proestrus, and lowest on estrus. Levels of opiate receptors in the sexual dimorphic region of the preoptic area are higher in females than in males (Hammer, 1984). In females the highest binding is on diestrus, and the lowest on proestrus.

Changes in the activity of endogenous opioid peptides in the preoptic area have been implicated in the hormone dependent stimulation of luteinizing hormone secretion (Allen and Kalra, 1986; Kalra and Kalra, 1983; 1984; Lustig, Pfaff and Fishman, 1988). Progesterone decreases the inhibitory influence of endogenous opiates on LH secretion (Gabriel, Simpkins and Kalra, 1983). The decreasing opiate tone in the preoptic area is thought to increase LHRH release through increased norepinephrine or epinephrine release (Kalra and Kalra, 1983).

In the work presented here, opiate regulation of sexual behavior was examined. The preoptic area was found to be an

important site for the regulation of lordosis by opiates. The behavioral actions of opiates were also found to require an intact serotonergic system. Opiates also modulated the turnover of serotonin, a neurotransmitter known to act in the preoptic area to regulate lordosis behavior. The decrease in opiate receptors in the POA after serotonergic lesions provides further evidence for interactions between opiates and serotonin in the preoptic area, and suggest that opiate receptors may be located on serotonergic terminals in the preoptic area. Our model for opiate regulation of lordosis has been elaborated to show the neuroanatomical specificity of opiate actions on serotonergic systems (Figure 21).

These data strongly suggest that alterations in serotonin turnover in the preoptic area are important for the mediation of lordosis by opiates. Two aspects of opiate regulation of lordosis and the role of serotonergic systems need to be further investigated. The role of the different classes of opiate peptides in the regulation of behavior and serotonin turnover needs to be examined (Pfaus and Grozalka, 1987b), concentrating on the actions of opiates in the preoptic area. The role of opiate-serotonergic interactions in the preoptic area in the regulation of lordosis should be investigated by examining the action of treatments with both serotonergic and opiate drugs on behavior, preferably after infusion into the preoptic area.

In these experiments, the neurochemical regulation of behavior was studied. Lordosis, sexual behavior in the female rat, was used as the model system. Lordosis has been used here and by other investigators to study hormonal and neurochemical regulation of a complex behavior. The work presented here extends previous investigations of the opiate regulation of lordosis. In addition, there is evidence that two previously described systems, serotonin and endogenous opiates, may in fact be working in concert to regulate behavior. Future studies will further examine interactions between these systems in the regulation of lordosis, with the goal being a more complete understanding of the role of specific neurotransmitter systems in controlling complex behaviors in mammals.

IX. METHODS

A. OVARIECTOMY

Adult female rats (200-250 grams, Charles River, Wilmington, Mass.) were purchased. Long-Evans hooded rats were used in the experiments examining the effects of intracranial infusion or implantation of opiates on sexual behavior. In all other experiments, female Sprague-Dawley rats were used. Rats were bilaterally ovariectomized under ether anesthesia at least five days before further treatments. Rats were maintained on a reversed 14:10 hour light:dark cycle, and given lab chow and water ad libitum.

B. SEXUAL BEHAVIOR TESTING

Sexual behavior was tested using male rats. Stud males and experimental subjects were housed in the same room, which is maintained on a reversed light:dark cycle (14 hour dark:10 hour light). The testing room was dimly lit with a red light. As often as possible, testing was done in the middle third of the dark part of the light cycle.

The testing arena was a glass aquarium (30 cm x 50 cm), with corn cob bedding on the floor. The male rat was placed in the aquarium for several minutes. Three aquaria were used at a time, with a single male rat in each aquarium. Female rats to be tested were placed in the aquarium with the males and rotated between the aquaria. The female rats were mounted ten times by the male rats during the test.

The rating system of Hardy and DeBold (1971) was used (Figure 1). For each mount, the lordosis of the female rat was rated on a scale of 0 to 3, where 0 = no lordosis, 1 = marginal lordosis, 2 = normal lordosis, and 3 = exagerated lordosis with extreme dorsoflexion (Hardy and Debold, 1971). Lordosis quotients and quality scores were calculated.

$$\text{Lordosis Quotient} = \frac{(\text{Number of lordosis}) \times 100}{(\text{Number of mounts})}$$

Quality Score = Mean lordosis score for the ten mounts

C. INTRACRANIAL CANNULAE

Three days after ovariectomy, bilateral stainless steel cannulae (Plastic Products, Roanoke, Va.) were implanted stereotaxically just dorsal to the medial preoptic area or just dorsal to the lateral portion of the ventromedial nucleus of the hypothalamus (Luine and Fischette, 1982). The cannulae consisted of 23 G outer cannulae, through which 28 G inner cannulae could be lowered. Inner cannulae extended 1 mm past the end of the outer cannulae. The coordinates for the VMN were 2.2 mm posterior to bregma, approximately 0.7 mm lateral to the midline, and 8.3 mm from the top of the skull. For the POA, the coordinates were 0.8 mm posterior to bregma, 0.7 mm lateral, and 8.3 mm ventral from the top of the skull. Dummy inner cannulae

were immediately inserted into the guide cannulae, and the animals were one week for recovery before behavior testing.

For studies examining the effect of opiates in the VMN, the inner cannulae were filled with crystalline naltrexone or morphine by tamping the drug into the lumen of the tube. Inner cannulae were then inserted into the guide cannulae, and sexual behavior was tested after 1, 2 and 3 hours.

Naltrexone (0.2 or 6 ug), morphine (4 ug), or saline were infused into the preoptic area. One ul of vehicle was infused over three minutes, and the infusion cannulae were left in place for three minutes to reduce leakage. The dummy inner cannulae were then replaced. Sexual behavior was tested 20 and 60 minutes aftere the infusion.

D. SEROTONERGIC LESIONS

Serotonergic axons in the brain were lesioned with the selective neurotoxin 5,7-dihydroxytryptamine (5,7-DHT). 5,7-DHT is taken up into serotonergic terminals by the specific neuronal uptake system for serotonin. The selectivity of the lesion can be improved by prior treatment with desipramine (Breese and Cooper, 1975). Desipramine inhibits the neuronal uptake of norepinephrine, and thereby inhibits the uptake of 5,7-DHT into noradrenergic terminals.

Ovariectomized female rats were anesthetized with Ketalar (Ketamine, mg i.p.) and Rompun (Xylazine, 1 mg i.m.), and placed in a Kopf stereotaxic apparatus. The top

of the head was swabbed with a 0.13% solution of Zephiran chloride (Winthrop Breon). The skin was open and the skull exposed. The incisor bar of the stereotaxic apparatus was adjusted so that the top of the skull was flat. 5,7-DHT was dissolved in saline containing 0.2 mg/ml ascorbic acid. Rats were injected with desipramine approximately one hour before intracranial infusion of the toxin (Breese and Cooper, 1975.)

a. Serotonergic lesions

In order to lesion serotonergic terminals throughout the brain, 5,7-DHT (100 ug x 2) was infused bilaterally into the lateral ventricles. Rats were injected with a higher dose of desipramine, since preliminary studies showed that the lower dose was not completely effective in protecting noradrenergic terminals after intraventricular infusion of a higher dose of the toxin (25 mg/kg, also see Fischette, Nock and Renner, 1987). The coordinates for the injection sites were 0.2 mm anterior to bregma, 3.6 mm ventral to the top of the brain, and 1 mm to both sides. The dissolved toxin (5 ul each side) was injected slowly over three minutes, and the needle was left in the brain for three minutes after the injection to minimize leakage back through the needle track.

b. Hypothalamic serotonergic lesions

In studies examining the effects of opiates on sexual behavior in rats with hypothalamic serotonergic lesions,

5,7-DHT was infused into the lateral hypothalamus (coordinates from lambda: 4.5 mm anterior; 0.6 mm lateral, and 8.5 mm ventral) using a 1 ul Hamilton syringe (Luine, Frankfurt, Rainbow, Biegon and Azmitia, 1983). A solution of 5,7-DHT (5 ug in 400 nl saline) was slowly injected over five minutes. Following injection, the syringe was left in place for an additional three minutes. Desipramine (10 mg/kg, Sigma) was injected one hour prior to the infusion of 5,7-DHT to protect noradrenergic terminals. Sham controls were injected with 400 nl of saline solution containing ascorbic acid at the same coordinates and over the same time period as the 5,7-DHT treated females. The animals were then housed singly.

E. PALKOVITS PUNCH TECHNIQUE

The punch-out method, originated by M. Palkovits (1973), allows the discrete sampling of specific nuclei within the brain. This method is combined with HPLC to measure levels of monoamines in discrete areas of the brain important for the regulation of female sexual behavior. The method consists of removing, blocking and freeze-mounting fresh, unperfused brain on cryostat chucks. Then 300 um sections are serially made and freeze-thaw mounted on microscope slides. The tissue sections are stored in slide boxes in a Revco freezer (-80° C) until sampling and analysis is complete.

Punching of samples is done under a dissecting microscope equipped with a constant temperature (-15° C) fluid cooled microscope stage. Hollow, stainless steel needles of 500 um in diameter are used to punch nuclei. The location of nuclei are determined by their relationship to various landmarks such as fiber tracts and the ventricles.

After punching, the tissue is expelled out of the needle with a stainless steel into a Sarstedt tube containing buffer for analysis of monoamines by HPLC.

F. HPLC ANALYSIS OF MONOAMINE LEVELS

The method of analysis of monoamines that was used in these experiments was developed by Renner and Luine (1984). Levels of norepinephrine, dopamine, serotonin and 5-hydroxyindole acetic acid can be simultaneously determined in microdissected brain nuclei from a single animal.

The chromatographic system consisted of a Waters Associates, Inc., Model 710B WISP automatic sample injector, a Model M45 pump, pulse dampeners, guard column packed with C18/corasil and a C18 uBondapack radially compressed column in a Z-module. During the work described here, several changes were made to the system. We switched to a C18 Novapack radial compression column, and Waters Guard-Pak precolumn. The Novapack column provides better peak resolution, which allows for shorter run times. A Waters Model 590 pump replaced the M45 pump. Electrochemical

detection is provided by a glassy carbon electrode (TL-5, Bioanalytical Systems), using a Ag/AgCl electrode as reference. A potential of + 0.65 Volts was maintained using a LC/4B potentiostat (Bioanalytical Systems). Chromatograms are recorded on a Waters 730 Data Module set in the peak height mode at 1 Volt full scale deflection. The detector sensitivity was set at 0.5 or 1.0 nAmp full scale deflection.

The mobile phase consisted of 6.8 grams sodium acetate (Fisher, HPLC grade), 100 mgram of disodium EDTA (Fisher) and 0.85 grams sodium heptane sulfonate (Kodak Co.) dissolved in 970 ml of double distilled, deionized water. The pH was adjusted to 3.5 with glacial acetic acid, after which 40 ml of acetonitrile (Waters Associates) was added. The mobile phase was filtered under vacuum using a 0.2 um nylon filter (Rainin Instrument Co.), and degassed. The mobile phase was recirculated and changed after 1 to 2 months of continuous use. The pump flow rate for the Bondapak columns was 2 ml per minute and the back pressure was approximately 1200 psi. The flow rate using the Novapak columns was 1 ml per minute and the back pressure was 700 psi.

All solutions were prepared in double distilled, deionized water. Norepinephrine bitartrate, dopamine HCl, serotonin creatinine sulfate, 5-hydroxyindole acetic acid, and 5-hydroxytryptamine were obtained from Sigma Chemical

Company. The internal standard, α-methyl dopamine was the kind gift of Dr. W. Gall of Merck, Sharp and Dohme. Concentrated solutions of norepinephrine and dopamine were prepared in 0.1 N $HClO_4$. Serotonin, 5-HIAA and 5-HTP stock solutions were prepared in 0.9% saline. Concentrations of the monoamines in the stock solutions ranged from 0.5 to 1.0 x 10^{-3} M. The solutions are stable for three weeks when stored at 3°C. The external standards are prepared daily by pipetting 10 ul of the stock solutions into 100 ml of a sodium acetate solution (containing 3 g sodium acetate, 4.3 ml glacial acetic acid, adjusted to ph 5 and brought up to 1 liter).

The tissue was expelled into a 1.5 ml Starstedt tube containing approximately 7.5 x 10^{-8} M α-methyl dopamine in 60 ul of the sodium acetate solution. After freeze-thawing to disrupt the tissue and release the monoamines, samples were centrifuged at 15,000 g for 15 minutes. Two microliters of an ascorbate oxidase solution (1 mg/10 ml, Boehringer Mannheim or Sigma Chemical) were added to each tube to decrease the contribution of ascorbic acid to the solvent front. The supernatant was removed, and 40 ul were injected into the chromatographic system. The pellet was dissolved in 100 ul of 0.2 N NaOH. Protein measurement was done according to the method of Bradford (1976).

The system was calibrated by injecting 40 ul of the external standard solution. The pg/cm peak height was

determined from the mean peak heights of three chromatograms for the respective standards. The internal standard, -methyl dopamine, was injected three times to establish the peak height of the internal standard for percentage recovery. The internal standard also serves to correct for slight variations in injection volume and degradation of the sample during preparation.

The intra-assay variability was determined by measuring pooled punches taken randomly from tissue slices. The coefficient of variability was 3.4% for norepinephrine, 1.1% for dopamine, 5.9% for 5-HIAA, and 3.6% for serotonin (Renner and Luine, 1984). The assay was linear from 20 pg to 2 ng for norepinephrine, 30 pg to 2 ng for dopamine, 25 pg to 2 ng for 5-HIAA, and 40 pg to 2 ng for serotonin, which was sufficient for evaluation of monoamines in nuclei from individual animals. Evaluation of repeated injections from several punched tissue samples and standards showed that NE, DA, 5-HIAA, and serotonin are stable over an eight to nine hour period.

G. ALZET MINIPUMP INFUSION OF OPIATES

One week after ovariectomy, female rats (200 to 250 gm) were implanted with Alzet minipumps subcutaneously into the intrascapular space. The minipumps contained morphine sulfate (70 mg/ml saline), naltrexone hydrochloride (70 mg/ml saline) or saline. The rate of infusion was

approximately 1 ul/hour. The doses of morphine sulfate and naltrexone hydrochloride were approximately 0.35 ug/kg/hour.

H. RADIOIMMUNOASSAY FOR LUTEINIZING HORMONE

Animals were decapitated and the trunk blood was collected for assay of LH as previously described (Krey and Parsons, 1982; Renner, Krey and Luine, 1987). Serum was separated by centrifugation and stored frozen until use. Levels of LH were measured by radioimmunoassay (Niswender, Midgler, Monroe and Reichert, 1968). This assay employed an antisera to ovine LH (GDN No. 15), ^{125}I-ovine tracer (LER1056-C_2) and a rat LH standard (NIAMDA-RP-1). LH values are expressed as ng NIAMDA RP-1/ ml serum.

I. AUTORADIOGRAPHY

The brains were removed, frozen in 2-methylbutane (-30°C), and stored at -70°C. Serial sections were cut at 14 um using a cryostat at -12°C. Sections were thaw-mounted onto subbed slides, vacuum dessicated for two hours at -5°C, and stored at -70°C.

a. Serotonin-1 receptor autoradiography

Brain sections were preincubated for 30 minutes in 170 mM Tris buffer (pH 7.6) containing 4 mM $CaCl_2$. Sections were incubated for 1 hour in the same buffer containing 2.5 nM ^{3}H-serotonin (24 Ci/mmol, NEN), 10 uM pargyline

(Sigma), and 1 uM fluoxetine (Lilly), the serotonin reuptake blocker. Nonspecific binding was assessed in adjacent sections by the addition of 1 uM unlabeled serotonin. After incubation, sections were washed for 5 minutes at 4°C in preincubation buffer, dipped in ice cold distilled water, and dried.

b. Opiate receptor autoradiography

For opiate receptor binding (Tempel and Zukin, 1987), brain sections were preincubated in 50 mM Tris·HCl buffer, pH 7.4, for 30 minutes at 37°C to facilitate removal of bound endogenous ligand from the receptors. Mu opiate receptors were labeled by incubating for 1 hour at 4°C in 50 mM Tris·HCl (pH 7.4) containing 5 nM ^{3}H[D-Ala^{2},N-MetPh^{4},-Gly-ol^{5}]enkephalin. For delta receptor binding, brain sections were incubated with 25 nM ^{3}H[D-Pen^{2},D-Pen^{5}]-enkephain in the same buffer for three hours. Nonspecific binding was assessed by incubating adjacent sections under the same conditions containing a 1000-fold excess of nonlabeled ligand.

c. Analysis

Dried sections and tritium standards (Amersham) were opposed to LKB Ultrofilm. Binding was analyzed with a DUMAS computer-assisted densitometer that converted gray levels to femtomoles/ mg tissue wet weight using the standard curve derived from tritium standards

J. STATISTICS

Lordosis quotients and quality scores are not normally distributed data, and require the use of nonparametric statistics. The Mann-Whitney U-test was used to compare two treatment groups. In cases where there were more than two groups, we used the Kruskal-Wallis test, which is the nonparametric equivalent to the one-way analysis of variance. Differences between groups were then tested using the Mann-Whitney U-test. For the measurement of sexual behavior after infusion of opiates into the brain, each animal was tested several times after the infusion. This data was analyzed using a repeated measures design analysis of variance. The program "SYSTAT" was used on an IBM XT computer for the analyses.

Levels of monoamines in the brain and the levels of neurotransmitter receptors were normally distributed. Differences between two groups were analyzed by t-test. Experiments with three treatment groups were initially analyzed by one-way analysis of variance. If this test showed a significant effect of the treatments, differences between groups were tested using Neuman-Keuls post-hoc test.

In the experiments measuring 5-HIAA levels after naltrexone or morphine, the initial statistical analysis was a one-way ANOVA. Differences between the treatment groups and the saline control were then analyzed by Dunnet's multiple T test.

With the "pargyline" method and the "AMPT" method, the rate of turnover is derived from the slope of the change in monoamine levels. Serotonin turnover is estimated from the rate of increase in serotonin levels after the inhibition of monoamine oxidase by pargyline. Catecholamine turnover is estimated from the decrease in the logarithm of the catecholamine levels after the inhibition of catecholamine synthesis by alpha-methyl-p-tyrosine. The slope of the line is the rate constant. Slopes of the lines were analyzed by least-squares linear regression analysis (Zar, 1974), and a two-tailed t-test was used for the statistical comparison of slopes. The turnover rate of the catecholamines is the rate constant times the initial concentration. The standard deviation of the turnover rate (Rance, Wise, Selmanoff and Barraclough, 1981) is:

$$SD_K = \tfrac{1}{4}(k)^2(SD_{[CA]})^2 + 2(k)[CA](\text{covariance}) + (SD_k)^2[CA]^2\P$$

where [CA] is the initial catecholamine concentration.

To determine the significance of differences between catecholamine turnover rates, the Z value was calculated and entered on a table of standard normal distribution (Rance, Wise, and Selmanoff, Barraclough, 1981). A computer program in Applesoft Basic was written to aid in the analysis of these turnover experiments. This program is included in the appendix.

X. APPENDIX

PROGRAM FOR THE ANALYSIS OF MONOAMINE TURNOVER EXPERIMENTS
Written in Applesoft Basic

This program was used to determine rate constants and monoamine turnover rates for norepinephrine, dopamine and serotonin.

```
5   HOME
10  DIM X(40),Y(40)
20  INPUT "AREA OF BRAIN ";A1$
30  INPUT "NEUROCHEMICAL ";A2$
40  INPUT "TREATMENT     ";A3$
50  HOME : PRINT : PRINT : PRINT : PRINT
60  PRINT "   TYPE IN NUMBER OF ANIMALS": PRINT "   AND TIME
     FOR EACH GROUP"
70  FOR I = 1 TO 3
75  PRINT : PRINT
80  PRINT "GROUP #";I;: INPUT " N: ";A(I)
90  PRINT "      ";: INPUT "TIME: ";T(I)
100  NEXT I
110  PRINT : PRINT : PRINT "ANY CHANGES? ";: GET Z$
120  IF Z$ > "O" THEN 50
130  LET N = A(1) + A(2) + A(3)
135  HOME
140  FOR I = 1 TO N
150  INPUT "Y = ";Y(I)
155  PRINT
160  NEXT I
170  FOR I = 1 TO A(1):X(I) = T(1): NEXT I
180  FOR I = A(1) + 1 TO A(1) + A(2):X(I) = T(2): NEXT I
190  FOR I = N + 1 - A(3) TO N:X(I) = T(3): NEXT I
200  HOME
210  PRINT  CHR$ (4);"PR#1"
220 C = 1:A = 1:B = A(1): GOSUB 1000: PRINT
230 CO = YM:SC = YE
240 C = 2:A = A(1) + 1:B = A(1) + A(2): GOSUB 1000
250 C = 3:A = N + 1 - A(3):B = N: GOSUB 1000
270  PRINT  CHR$ (4);"PR#O"
500  REM  MENU FOR SELECTIONS
510  HOME
515  PRINT : PRINT : PRINT
520  PRINT "          ";: INVERSE : PRINT "MENU": NORMAL
525  PRINT : PRINT
530  PRINT " 1) DATA PRINTOUT "
540  PRINT " 2) DATA CORRECTION "
550  PRINT " 3) LOG TRANSFORMATION "
560  PRINT " 4) LINEAR REGRESSION "
```

```
570  PRINT " 5) TURNOVER ANALYSIS "
573  PRINT " 6) RERUN PROGRAM "
575  PRINT " 7) END "
580  PRINT : INPUT "TYPE NUMBER ";Z
590  ON Z GOSUB 2000,3000,3500,4000,5000,800,4500
600  GOTO 510
800  REM : RERUN PROGRAM
810  REM : ** DISABLE GOSUB
820  HOME
830 R1 = 0:L = 0
840  GOTO 20
1000  REM : MEAN AND STANDARD ERROR
1010 YS = 0:Y2 = 0
1015  IF C = 3 AND A(3) = 0 THEN 1060
1020  FOR I = A TO B:YS = YS + Y(I):Y2 = Y2 + (Y(I) ^ 2):
     NEXT I
1030 YM = YS / (1 + B - A):YD =  SQR ((Y2 / (1 + B - A) - YM
     ^ 2))
1040 YE = YD / ( SQR (B - A))
1050  PRINT : PRINT : PRINT "GROUP #";C: PRINT "   MEAN =
     ";YM: PRINT "     SE = ";YE
1060  RETURN
2000  REM : DATA PRINTOUT
2005  PRINT  CHR$ (4);"PR#1"
2010  PRINT : PRINT
2015  PRINT : PRINT A1$: PRINT "   ";A2$: PRINT "   ";A3$
2018  PRINT
2020  FOR I = 1 TO N
2030  PRINT I,X(I),Y(I)
2040  NEXT I
2045  PRINT : PRINT : PRINT
2050  PRINT  CHR$ (4);"PR#0"
2060  RETURN
3000  REM : CORRECTION ROUTINE
3010  HOME
3020  INPUT "WHICH SAMPLE NEEDS CORRECTING ";Q
3030  IF Q > N THEN  GOTO 3010
3040  HOME : PRINT "SAMPLE #";Q: PRINT
3050  PRINT " X = ";X(Q)
3060  PRINT " Y = ";Y(Q)
3070  PRINT : PRINT : PRINT "INPUT NEW VALUES"
3080  INPUT " X = ";X(Q)
3090  INPUT " Y = ";Y(Q)
3100  PRINT : PRINT : INPUT "CORRECT ANOTHER SAMPLE ";Z$
3110  IF Z$ > "O" THEN 3010
3120  RETURN
3500  REM : LOG TRANSFORMATION
3502  HOME : PRINT : PRINT : PRINT : PRINT "       LOG
     TRANSFORMATION": PRINT : PRINT : PRINT "        ";:
     INVERSE : PRINT "WORKING": NORMAL
3520  FOR I = 1 TO N:Y(I) =  LOG (Y(I)): NEXT I:L = 1
```

```
3540  RETURN
4000  REM : LINEAR REGRESSION
4002  PRINT : PRINT : PRINT : PRINT : PRINT "      LINEAR
     REGRESSION": PRINT : PRINT : PRINT "          ";: FLASH
     : PRINT "WORKING": NORMAL
4005 X2 = 0:Y2 = 0:XY = 0:YS = 0:XS = 0:R1 = 1
4020  FOR I = 1 TO N:XS = XS + X(I):YS = YS + Y(I): NEXT I
4040 DF = N - 2:XN = XS / N:YN = YS / N
4050  FOR I = 1 TO N
4060 XD = X(I) - XN:YD = Y(I) - YN
4070 X2 = X2 + XD ^ 2:Y2 = Y2 + YD ^ 2:XY = XY + XD * YD
4080  NEXT I
4090 K = XY / X2:SS = Y2 - (XY ^ 2) / X2
4100  PRINT  CHR$ (4);"PR#1"
4110  PRINT "LINEAR REGRESSION"
4120  PRINT : PRINT A1$: PRINT "   ";A2$: PRINT "   ";A3$
4130  PRINT : PRINT : PRINT "X2 = ";X2: PRINT "Y2 = ";Y2:
     PRINT "XY = ";XY: PRINT "SS residual = ";SS: PRINT
4140  PRINT "SLOPE = ";K
4150  PRINT " S.E. = "; SQR (SS / X2 / DF)
4170  PRINT  CHR$ (4);"PR#0"
4180  RETURN
4500  REM : END ROUTINE
4505  HOME : END
5000  REM : TURNOVER
5001  HOME
5002  IF R1 = 0 THEN  GOTO 5200
5004  IF L = 0 THEN  GOTO 5400
5010 SO = SC *  SQR (A(1))
5020 CV = XY / DF:SX = SS / DF
5030 SE =  SQR (SX / X2):SD =  SQR (N) * SE
5040 Z1 = (K * SO) ^ 2:Z2 = 2 * CO * CV * K:Z3 = (SD * CO) ^
     2
5050 Z =  SQR (Z1 + Z2 + Z3)
5060  PRINT  CHR$ (4);"PR#1"
5070  PRINT : PRINT "SLOPE = ";K;" + ";SE
5080  PRINT : PRINT "Co = ";CO;" + ";SC
5090  PRINT : PRINT : PRINT "TURNOVER RATE = ";K * CO
5100  PRINT "STANDARD ERR  = ";Z /  SQR (DF)
5110  PRINT "DEG OF FREEDOM= ";DF
5120  PRINT  CHR$ (4);"PR#0
5130  RETURN
5200  PRINT "LINEAR REGRESSION FIRST": GOTO 5410
5400  PRINT "LOG TRANSFORMATION FIRST"
5410  FOR I = 1 TO 500: NEXT I
5420  GOTO 5130
```

XI. REFERENCES

Ahlenius, S., A. Fernandez-Guasti, S. Hjorth, and K. Larsson. (1986) Suppression of lordosis behavior by the putative 5-HT agonist 8-OH-DPAT in the rat. Eur. J. Pharmacol. 124:361-363.

Akaishi, T., and Y. Sakuma. (1986) Projections of oestrogen-sensitive neurones from the ventromedial nucleus of the female rat. J. Physiol. 372:207-220.

Allen, D.L., K.J. Renner, and V.N. Luine. (1985) Naltrexone facilitation of sexual receptivity in the rat. Horm. Behav. 19:98-103.

Allen, L.G., and S.P. Kalra. (1986) Evidence that a decrease in opioid tone may evoke preovulatory luteinizing hormone release in the rat. Endocrinology 118:2375-2381.

Anden, N.-E., H. Corrodi, A. Dahlstrom, K. Fuxe and T. Hokfelt (1966) Effects of tyrosine hydroxylase inhibition on the amine levels of central monoamine neurons. Life Sciences 5:561-568.

Anden, N.-E., H. Corrodi, K. Fuxe and U. Ungerstedt. (1971) Importance of nervous impulse flow for the neuroleptic induced increase in amine turnover in central dopamine neurons. Eur. J. Pharmacol. 15:193-199.

Atweh, S.F., and M.J. Kuhar. (1983) Distribution and physiological significance of opioid receptors in the brain. Br. Med. Bull. 39:47-52.

Axelrod, J., and R. Tomchick. (1958) Enzymatic o-methylation of epinephrine and other catechols. J. Biol. Chem. 233:702-705.

Azmitia, E.C. (1978) The serotonin-producing neurons of the midbrain median and dorsal raphe nuclei. In: Handbook of Psychopharmacology, Vol 9, edited by Iverson, Iverson, and Snyder, Plenum Press, New York, pp. 233-314.

Azmitia, E.C., P. Hess, and D. Reis. (1970) Tryptophan hydroxylase changes in the midbrain of the rat after chronic morphine administration. Life Sciences 9:633-637.

Barden, N., Y. Merand, D. Rouleau, M. Garon and A. Dupont. (1981) Changes in the beta-endorphin content of discrete hypothalamic nuclei during the estrous cycle of the rat. Brain Res. 204:441-445.

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

Bast, J.D., C. Hunts, K.J. Renner, R.K. Morris and D.M. Quadagno. (1987) Lesions in the preoptic area suppressed sexual receptivity in ovariectomized rats with estrogen implants in the ventromedial hypothalamus. Brain Res. Bull. 18:153-158.

Bernardis, L.L., and L.L. Bellinger. (1987) The dorsomedial hypothalamic nucleus revisited: 1986 update. Brain Res. Rev. 12:321-381

Bicknell, R.J. (1985) Endogenous opioid peptides and hypothalamic neuroendocrine neurones. J. Endo. 107:437-446.

Blaustein, J.D. (1985) Noradrenergic inhibitors cause accumulation of nuclear progestin receptors in guinea pig hypothalamus. Brain Res. 325:89-98.

Blaustein, J.D., T.J. Brown and E.S. Swearengen. (1986) Dopamine-beta-hydroxylase inhibitors modulate the concentration of functional estrogen receptors in female rat hypothalamus. Neuroendocrinology 43:150-158.

Bonnet, K.A., P. Alpert, and S. Klinerock. (1978) Narcotic antagonists increase pain sensitivity in rats. In: Developments in Neuroscience, Vol 4. Edited by van Ree and Terenius. Elselvier/North-Holland, Amsterdam/New York, pp. 159-160.

Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.

Breese, G.R., and B.R. Cooper. (1975) Behavioral and biochemical interactions of 5,7-dihydroxytryptamine with various drugs when administered intracisternally to adult and developing rats. Brain Res. 98:517-527.

Brodie, B.B., E. Costa, A. Dlabac, N.H. Neff and H.H. Smookler. (1966) Application of steady state kinetics to the estimation of synthesis rate and turnover time of tissue catecholamines. J. Pharmacol. Exp. Therap. 154:493-498.

Bruni, J.F., D. Van Vugt, S. Marshall, and J. Meites. (1977) Effects of naloxone, morphine and methionine enkephalin on serum prolactin, luteinizing hormone, follicle

hormone, thyroid stimulating hormone and growth hormone. Life Sciences 21:461-466.

Butcher, R.L., W.E. Collins, and N.W. Fugo. (1974) Plasma concentration of LH, FSH, prolactin, progesterone and estradiol-17 throughout the 4-day estrous cycle of the rat. Endocrinology 94:1704-1708.

Caggiula, A.R., S.M. Antelman, L.A. Chiodo and C.G. Lineberry. (1979) Brain dopamine and sexual behavior: Psychopharmacological and electrophysiological evidence for an antagonism between active and passive components. In: Catecholamines: Basic and Clinical Frontiers. Editted by E. Usdin, I. Kopin and J. Barchas. Plenum Press, New York, pp. 1765-1767.

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

Caldwell, J.D. and L.G. Clemens. (1986) Norepinephrine infusions into the medial preoptic area inhibit lordosis behavior. Pharmacol. Biochem. Behav. 24:1015-1023.

Carrer, H.F. (1978) Mesencephalic participation in the control of sexual behavior in the female rat. J. Comp. Physiol. Psychol. 92:877-887.

Cicero, T.J., T.M. Badger, C.E. Wilcox, R.D. Bell, and E.R. Meyer. (1977) Morphine decreases luteinizing hormone by an action on the hypothalamic-pituitary axis. J. Pharm. Exp. Therap. 203:548-555.

Clark, A.S., H.H. Feder and E.J. Roy. (1985) $Alpha_1$-noradrenergic receptor blockade decreases nuclear estrogen receptor binding in guinea pig hypothalamus and preoptic area. Brain Res. 330:197-198.

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

Cooper, J.R., F.E. Bloom, and R.H. Roth. (1978) The Biochemical Basis of Neuropharmacology. Oxford University Press, New York.

Costa, E. and N.H. Neff. (1970) Estimation of turnover rates to study the metabolic regulation of the steady state

level of neuronal monoamines. In: Handbook of Neurochemistry, edited by A. Lajtha. New York: Plenum Press, pp. 45-90.

Crowley, W.R. (1982) Effects of ovarian hormones on norepinephrine and dopamine turnover in individual hypothalamic and extrahypothalamic nuclei. Neuroendo. 34:381-386.

Crowley, W.R., T.L. O'Donohue, H. Wachslicht, and D.M. Jacobowitz. (1978) Effects of estrogen and progesterone on plasma gonadotropins and on catecholamine levels and turnover in discrete brain regions of ovariectomized rats. Brain Research 154:345-357.

Cuello, A.C. (1983) Central distribution of opioid peptides. Br. Med. Bull. 39:11-16.

Davis, G.A., and R. Kohl. (1977) The influence of alpha-receptors on lordosis in the female rat. Pharmacol. Biochem. Behav. 6:47-53.

Davis, G.A., and R. Kohl. (1978) Biphasic effects of the antiserotonergic methysergide on lordosis in rats. Pharmacol. Biochem. Behav. 9:487-491.

Diez-Guerra, F.J., S. Augwood, P.C. Emson, and R.G. Dyer. (1987) Opioid peptides inhibit the release of noradrenaline from slices of rat medial preoptic area. Exp. Brain Res. 66:378-384.

Dorner, G., F. Docke, and G. Hinz. (1969) Homo- and hypersexuality in rats with hypothalamic lesions. Neuroendocrinology 4:20-24.

Dupont, A., N. Barden, L. Cusan, Y. Merand, F. Labrie, and H. Vaudry. (1980) Beta-endorphin and met-enkephalins: Their distribution, modulation by estrogens and haloperidol, and role in neuroendocrine control. Fed. Proc. 39:2544-2550.

Duval, C. and E. Mignot (1985) Effects of rat ovariectomy on CSF monoamine metabolite levels and elimination. J. Neurochem. 44:1312-1314.

Eisenberg, R.M. (1980) Effects of naloxone on plasma corticosterone in the opiate-naive rat. Life Sci. 26:935-943.

Espino, C., M. Sano, and G.N. Wade. (1975) Alpha-methyltryptamine blocks facilitation of lordosis by

progesterone in spayed, estrogen-primed rats. Pharmacol. Biochem. Behav. 3:557-559.

Etgen, A.M., and R.J. Barfield. (1986) Antagonism of female sexual behavior with intracerebral implants of antiprogestin RU 38486: Correlation with binding to neural progestin receptors. Endocrinology 119:1610-1617.

Everitt, B.J., K. Fuxe, and T. Hokfelt. (1974) Inhibitory role of dopamine and 5-hydroxytryptamine in the sexual behavior of female rats. Eur. J. Pharmacol. 29:187-191.

Everitt, B.J., K. Fuxe, and T. Hokfelt. (1975) Serotonin, catecholamines and sexual receptivity of female rats. Pharmacological findings. J. Pharmacol. 6:269-276.

Everitt, B.J., K. Fuxe, T. Hokfelt, and G. Jonsson. (1975) Role of monoamines in the control by hormones of sexual receptivity in the female rat. J. Comp. Physiol. Psychol. 89:556-572.

Feder, H.H. (1981) Estrous cyclicity in mammals. In: Neuroendocrinology of Reproduction. Edited by N. Adler, Plenum Press, New York, pp. 279-348.

Fernandez-Guasti, A., K. Larson, and C. Beyer. (1985a) Prevention of progesterone-induced lordosis behavior by alpha or beta adrenergic antagonists in ovariectomized estrogen-primed rats. Pharmacol. Biochem. Behav. 22:279-282.

Fernandez-Guasti, A., K. Larson, and C. Beyer. (1985b) Potentiative action of alpha- and beta-adrenergic receptor stimulation in inducing lordosis behavior. Pharmacol. Biochem. Behav. 22:613-617.

Fernandez-Guasti, A., S. Ahlenius, S. Hjorth, and K. Larsson. (1987) Separation of dopaminergic and serotonergic inhibitory mechanisms in the mediation of estrogen-induced lordosis behavior in the rat. Pharmacol. Biochem. Behav. 27:93-98.

Fischette, C.T., B. Nock, and K.J. Renner. (1987) Effects of 5,7-dihydroxytryptamine on serotonin$_1$ and serotonin$_2$ receptors throughout the rat central nervous system using quantitative autoradiography. Brain Res. 421:263-279.

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

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

Forman, L.J., D.E. Marquis, and R. Stevens. (1985a) The effect of chronic estrogen treatment on immunoreactive beta-endorphin levels in intact female rats. Proc. Soc. Exp. Biol. Med. 179:365-372.

Frankfurt, M., J.M. Lauder, and E.C. Azmitia. (1981) The immunocytochemical localization of serotonergic neurons in the rat hypothalamus. Neurosci. Lett. 24:227-232.

Frankfurt, M, K. Renner, E. Azmitia, and V.N. Luine. (1985) Intrahypothalamic 5,7-dihydroxytryptamine: Temporal analysis of effects on 5-hydroxytryptamine content in brain nuclei and on facilitated lordosis behavior. Brain Res. 340:127-133.

Gabriel, S.M., and J.W. Simpkins. (1983) Effects of a sustained-release naloxone pellet on luteinizing hormone secretion in female rats. Neuroendocrinology 37:342-348.

Gabriel, S.M., J.W. Simpkins, and S.P. Kalra. (1983) Modulation of endogenous opioid influence on luteinizing hormone secretion by progesterone and estrogen. Endocrinology 113:1806-1811.

Glaser, J.H., B.S. Rubin, and R.J. Barfield. (1983) Onset of the receptive and proceptive components of feminine sexual behavior in rats following the intravenous administration of progesterone. Hormones Behav. 17:18-27.

Gorzalka, B., and G. Lester. (1987) Oxytocin-induced facilitation of lordosis behavior in rats is progesterone- dependent. Neuropeptides 10:55-65.

Gunnet, J.W., K.J. Lookingland, and K.E. Moore. (1986) Comparison of the effects of castration and steroid replacement on incertohypothalamic dopaminergic neurons in male and female rats. Neuroendocrinology 44:269-275.

Hagan,R.M. and I.E. Hughes. (1984) Opioid receptor sub-types involved in the control of transmitter release in cortex of the brain of the rat. Neuropharm. 23:491-495.

Hamburg, M., and J.F. Tallman. (1981) Chronic morphine administration increases the apparent number of alpha$_2$-adrenergic receptors in rat brain. Nature 291:493-495.

Hammer, R.P., Jr. (1984) The sexually dimorphic region of the preoptic area in rats contains denser opiate binding sites in females. Brain Res. 308:172-176.

Hardy, D.F., and J.F. DeBold. (1971) Effects of mounts without intromissions upon the behavior of female rats during the onset of estrogen-induced heat. Physiol. Behav. 7:643-645.

Harlan, R., B. Shivers, and D.W. Pfaff. (1983) Midbrain microinfusions of prolactin increase the estrogen-dependent behavior, lordosis. Science 219:1451-1453.

Harlan, R.E., B.D. Shivers, G.J. Romano, R.D. Howells, and D.W. Pfaff. (1987) Localization of preproenkephalin mRNA in the rat brain and spinal cord by in situ hybridization. J. Comp. Neurol. 258:159-184.

Hart, B.L. (1969) Gonadal hormones and sexual reflexes in the female rat. Hormones Behav. 1:65-71.

Hashimoto, K., S. Suemaru, T. Hattori, T. Takao, H. Inoue, M. Sugawara, J. Kageyama, and Z. Ota. (1986) Effects of ($D\text{-}Ala^2$, Met^5)-enkephalinamide and naloxone on ACTH and corticosterone secretion. Endocrinol. Japon. 33:813-820.

Henrik, E., and A.A. Gerall. (1976) Facilitation of receptivity in estrogen-primed rats during successive mating tests with progestins and methysergide. J. Comp. Physiol. Psychol. 6:590-600.

Hetta, J. (1976) Effects of morphine and naltrexone on female sexual behavior. Acta Physiol. Scand. Suppl. 440:107.

Hlinak, Z. (1987) Lisuride inhibits temporarily sexual behavior in female rats. Pharm. Biochem. Behav. 27:211-215.

Hokfelt, T., R. Elde, O. Johansson, L. Terenius, and L. Stein. (1977) The distribution of enkephalin-immunoreactive cell bodies in the rat central nervous system. Neurosci. Lett. 5:25-31.

Hughes J., and H.W. Kosterlitz. (1983) Introduction. Br. Med. Bull. 39:1-3.

Hughes, J. (1983) Biogenesis, release and inactivation of enkephalins and dynorphins. Br. Med. Bull. 39:17-24.

Jezova, D., M. Vigas, and J. Jurcovicova. (1982) ACTH and corticosterone response to naloxone and morphine in normal,

hypophysectomized and dexamethasone-treated rats. Life. Sci. 31:307-314.

Jirikowski, G.F., I. Merchenthaler, J.E. Rieger and W.E. Stumpf. (1986) Estradiol target sites immunoreactive for beta-endorphin in the arcuate nucleus of rat and mouse hypothalamus. Neurosci. Lett. 65:121-126.

Johnson, M.D. and W.R. Crowley. (1983) Acute effects of estradiol on circulating luteinizing hormone and prolactin concentrations and on serotonin turnover in individual brain nuclei. Endocrinology 113:1935-1941.

Johnson, M.D. and W.R. Crowley. (1984) Effects of opiate antagonists on serotonin turnover and on luteinizing hormones and prolactin secretion in estrogen- or morphine- treated rats. Neuroendocrinology 38:322-327.

Johnson, M.D., and W.R. Crowley. (1986) Role of central serotonin systems in the stimulatory effects of ovarian hormones and naloxone on luteinizing hormone release in female rats. Endocrinology 118:1180-1186.

Johnston, C.A., and K.E. Moore. (1983) The effect of morphine on 5-hydroxytryptamine synthesis and metabolism in the striatum, and several discrete hypothalamic regions of the rat brain. J. Neural Transmission 57:65-73.

Kalra, S.P., and P.S. Kalra. (1983) Neural regulation of luteinizing hormone secretion in the rat. Endo. Rev. 4:311-351.

Kalra, S.P., and P.S. Kalra. (1984) Opioid-adrenergic-steroid connection in regulation of luteinizing hormone secretion in the rat. Neuroendocrinology 38:418-426.

Kiang, J.G., W.L. Dewey and E.T. Wei. (1983) Tolerance to morphine bradycardia in the rat. J. Pharmacol. Exper. Therap. 226:187-191.

King, T.S., R.W. Steger, and W.W. Morgan. (1986) Effect of ovarian steroids to stimulate region-specific hypothalamic 5-hydroxytryptamine synthesis in ovariectomized rats. Neuroendocrinology 42:344-350.

Knapp, S., and A.J. Mandell. (1972) Narcotic drugs: Effect on the serotonin biosynthetic systems of the brain. Science 177:1209-1211.

Kolta, M.G., K.F.A. Soliman, and B.B. Williams. (1986) Role of 5-hydroxytryptamine in the regulation of brain

neuropeptides in normal and diabetic rat. Hormone Res. 23:112-121.

Koulo, M., J.M. Saavedra, N. Bjelogrlic, M. Niwa, H. Agren, and M. Linnoila. (1986) Serotonin turnover in discrete hypothalamic nuclei and mesencephalic raphe nuclei of young and adult spontaneously hypertensive rats. Brain Res. 379:257-263.

Kow, L.-M., and D.W. Pfaff. (1975) Induction of lordosis in female rats: Two modes of estrogen action and the effect of adrenalectomy. Hormones Behav. 6:259-276.

Kow, L.-M., M.O. Montgomery, and D.W. Pfaff. (1977) Effects of spinal cord transections on lordosis reflex in female rats. Brain Res. 123:75-88.

Krey, L.C. and B. Parsons. (1982) Charaterization of estrogen stimuli sufficient to initiate cyclic luteinizing hormone release in acutely ovariectomized rats. Neuroendocrinology 34:315-322.

Kubli-Garfias, C., and R.E. Whalen. (1977) Induction of lordosis behavior in female rats by intravenous administration of progestins. Hormones Behav. 9:380-386.

Kuhn, D.M., W.A. Wolf, and M.B.H. Youdim. (1986) Serotinin neurochemistry revisited: A new look at some new axioms. Neurochem. Int. 8:141-154.

Law, T., and W. Meagher. (1958) Hypothalamic lesions and sexual behavior in the female rat. Science 128:1626-1627.

Leadem, C.A., W.R. Crowley, J.W. Simpkins, and S.P. Kalra. (1985) Effects of naloxone on catecholamine and LHRH release from the perifused hypothalamus of the steroid-primed rat. Neuroendocrinology 40:497-500.

Levitt, M., S. Spector, A. Sjoerdsma, and S. Udenfriend. (1965) Elucidation of the rate-limiting step in norepinephrine biosynthesis in the perfused guinea-pig heart. J. Pharmacol. Exp. Therap. 148:1-7.

Lewis, M.E., H. Khachaturian and S.J. Watson. (1985) Combined autoradiographic-immunocytochemical analysis of opioid receptors and opioid peptide neuronal systems in the brain. Peptides Volume 6 (Supplement 1):37-47.

Lin, R., N. Neff, S. Ngai, and E. Costa. (1969) Turnover rates of serotonin and norepinephrine in brain of normal and pargyline-treated rats. Life Sciences 8:1077-1084.

Lindblom, C., G. Forsberg, and P. Sodersten. (1986) The effect of naloxone on sexual behavior in female rats depends on the site of injection. Neurosci. Lett. 70:97-100.

Llorens, C., M. Martres, M. Baudry, and J. Schwartz. (1978) Hypersensitivity to noradrenaline in cortex after chronic morphine: Relevance to tolerance and dependence. Nature 274:603-605.

Luine,V.N. and C.T. Fischette. (1982) Inhibition of lordosis behavior by intrahypothalamic implants of pargyline. Neuroendocrinology 34:237-244.

Luine, V.N., and C. Paden (1982) Effect of monoamine oxidase inhibition on female sexual behavior, serotonin levels and type A and B monoamine oxidase activity. Neuroendocrinology 34:245-251.

Luine, V.N., M. Frankfurt, T. Rainbow, A. Biegon, and E.C. Azmitia. (1983) Intrahypothalamic 5.7-dihydroxytryptamine facilitates feminine sexual behavior and decreases [^{3}H]imipramine binding and 5-HT uptake. Brain Research 264:344-348.

Luine, V.N., K.J. Renner, M. Frankfurt, and E.C. Azmitia. (1984) Facilitated sexual behavior reversed and serotonin restored by raphe nuclei transplanted into denervated hypothalamus. Science 226:1436-1439.

Luine, V.N., J.E. Thornton, M. Frankfurt and N.J. MacLusky. (1987) Effects of hypothalamic serotonin depletion on lordosis behavior and gonadal hormone receptors. Brain Res. 426:47-54.

Lustig, R., D. Pfaff, and J. Fishman. (1988) Opioidergic modulation of the oestradiol-induced LH surge in the rat: Roles of ovarian steroids. J. Endo. 116:55-69.

Martin, W.R., C.G. Eades, J.A. Thompson, R.E. Huppler and P.E. Gilbert. (1976) The effects of morphine and nalorphine-like drugs in the nondependent and the morphine-dependent chronic spinal dog. J. Pharmacol. Exper. Therap. 197:517-532.

Mathews, D., and D.A. Edwards. (1977) Involvement of the ventromedial and anterior hypothalamic nuclei in the hormonal induction of receptivity in the female rat. Physiol. Behav. 19:319-326.

McEwen, B.S., A. Biegon, P.G. Davis, L.C. Krey, V.N. Luine, M.Y. McGinnis, C.M. Paden, B. Parsons and T.C. Rainbow.

(1982) Steroid hormones: Humoral signals which alter brain cell properties and function. Recent Progress Hormone Research 38:41-92.

McGinty, J.F., and F.E. Bloom. (1983) Double immunostaining reveals distinctions among opioid peptidergic neurons in the medial basal hypothalamus. Brain Res. 278:145-183.

McKay, L., C. Bradberry, and A. Oke. (1984) Ascorbate oxidase speeds up analysis for catecholamines, indoleamines, and their metabolites in brain tissue. J. Chr. Biomed. Appl. 311:167-169.

Meites, J., J.F. Bruni, D.A. Van Vugt, and A.F. Smith. (1979) Relation of endogenous opioid peptides and morphine to neuroendocrine functions. Life Sciences 24:1325-1336.

Mendelson, S.D. and B.B. Gorzalka. (1985) A facilitatory role for serotonin in the sexual behavior of the female rat. Pharm. Biochem. Behav. 22:1025-1033.

Mendelson, S.D. and B.B. Gorzalka. (1986a) Effects of 5-HT_{1a} selective anxiolytics on lordosis behavior: Interactions with progesterone. Eur. J. Pharm. 132:323-326.

Mendelson, S.D. and B.B. Gorzalka. (1986b) 5-HT_1A receptors: Differential involvement in female and male sexual behavior in the rat. Physiol. Behav. 37:345-351.

Meyerson, B.J. (1964a) The effect of neuropharmacological agents on hormone-activated estrus behavior in ovariectomized rats. Arch. Int. Pharmacodyn. 150:4-33.

Meyerson, B.J. (1964b) Central nervous monoamines and hormone induced estrus behavior in the spayed rat. Acta Physiologica Scand. Vol. 63, Supplementum 241:3-32.

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

Meyerson, B. (1972) Latency between intravenous injection of progestins and the appearance of estrous behavior in estrogen-treated ovariectomized rats. Hormones Behav. 3:1-9.

Meyerson, B., and C.O. Malmnas. (1978) Brain monoamines and sexual behavior. In: Biological Determinants in Sexual Behavior. Editted by J. Hutchinson. Wiley, London, pp. 521-554.

Millard, S., E. Costa, and E. Gal. (1972) On the control of brain serotonin turnover rate by end product inhibition. Brain Research 40:545-551.

Moir, A.T.B. (1971) Interaction in the cerebral metabolism of the biogenic amines: Effect of intravenous infusion of l-tryptophan on the metabolism of dopamine and 5-hydroxyindoles in brain and cerebrospinal fluid. Br. J. Pharmac. 43:715-723.

Morgenroth, V.H., J.R. Walters and R.H. Roth. (1976) Dopaminergic neurons-alteration in the kinetic properties of tyrosine hydroxylase after cessation of impulse flow. Biochem. Pharm. 25:655-661.

Morrell, J.I., J.F. McGinty and D.W. Pfaff. (1985) A subset of beta-endorphin- or dynorphin-containing neurons in the medial basal hypothalamus accumulates estradiol. Neuroendocrinology 41:417-426.

Moss, R.L., and S.M. McCann. (1973) Induction of mating behavior in rats by luteinizing hormone-releasing factor. Science 181:177-179.

Myers, B.M., and M.J. Baum. (1979) Facilitation by opiate antagonists of sexual performance in the male rat. Pharmacol. Biochem. Behav. 10:615-618.

Nagatsu, T., M. Levitt, and S. Udenfriend. (1964) Tyrosine hydroxylase: The initial step in norepinephrine biosynthesis. J. Biol. Chem. 239:2910-2917.

Neff, N.H., and E. Costa. (1966) The influence of monoamine oxidase inhibition on catecholamine synthesis. Life Sciences 5:951-959.

Neff, N.H., and E. Costa. (1968) Application of steady-state kinetics to the study of catecholamine turnover after monoamine oxidase inhibition or reserpine administration. J. Pharmacol. Exp. Therap. 160:40-47.

Ngai, S.H., N.H. Neff, and E. Costa. (1968) Effect of pargyline treatment on the rate of conversion of tyrosine C^{14} to norepinephrine C^{14}. Life Sciences 7:847-855.

Nikolarakis, K., A. Pfeiffer, G.K. Stalla, and A. Herz. (1987) The role of CRF in the release of ACTH by opiate agonists and antagonists in rats. Brain Res. 421:373-376.

Niswender, G.D., A.R. Midgler,Jr., S.E. Monroe, and L.E. Reichert, Jr. (1968) Radioimmunoassay for rat luteinizing

hormone with anti-ovine LH serum and ovine LH-^{131}I. Proc. Soc. exp. Biol. Med. 128:897-900.

Nock, B., J.D. Blaustein, and H.H. Feder. (1981) Changes in noradrenergic transmission alter the concentration of cytoplasmic progestin receptors in hypothalamus. Brain Res. 207:371-396.

Nock, B., and H.H. Feder. (1981) Neurotransmitter modulation of steroid action in target cells that mediate reproduction and reproductive behavior. Neurosci. Biobehav. Rev. 5:437-447.

Nock, B., and H.H. Feder. (1984) Alpha-$_1$-noradrenergic regulation of hypothalamic progestin receptors and guinea pig lordosis behavior. Brain Res. 310:77-85.

O'Conner, L.H., and H.H. Feder. (1983) Effects of serotonin agonists on lordosis, myoclonus, and cytoplasmic progestin receptors in guinea pigs. Hormones Behav. 17:183-196.

Ostrowski, N.L., J.M. Stapleton, R.G. Noble, and L.D. Reid. (1979) Morphine and naloxone's effects on sexual behavior of the female golden hamster. Pharm. Biochem. Behav. 11:673-681.

Ostrowski, N.L., R.G. Noble, and L.D. Reid. (1981) Opiate antagonists and sexual behavior in female hamsters. Pharm. Biochem. Behav. 14:881-888.

Palkovits, M. (1973) Isolated removal of hypothalamic or other brain nuclei of the rat. Brain Res. 59:449-450.

Parenti, M., F. Tirone, V.R. Olgiati, and A. Groppetti. (1983) Presence of opiate receptors on striatal serotonergic nerve terminals. Brain Research 280:317-322.

Paris, C.A., J.A. Resko, and R.W. Goy. (1971) A possible mechanism for the induction of lordosis by reserpine in spayed rats. Biol. Reprod. 4:23.

Parsons, B., T.C. Rainbow, D.W. Pfaff, and B.S. McEwen. (1982) Hypothalamic protein synthesis essential for the activation of lordosis reflex in the female rat. Endocrinology 110:620-624.

Paterson, S.J., L.E. Robson and H.W. Kosterlitz. (1983) Classification of opioid receptors. Br. Med. Bull. 39:31-36.

Pazos, A. and J.M. Palacios. (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-1 receptors. Brain Res. 346:205-230.

Pazos, A., R. Cortes, and J.M. Palacios. (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotinin-2 receptors. Brain Res. 346:231-249.

Peroutka, S.J. (1986) Pharmacological differentiation and characterization of 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1C} binding sites in rat frontal cortex. J. Neurochem. 47:529-540.

Petraglia, F., V. Locatelli, F. Facchinetti, M. Bergamaschi, A.R. Genazzani and D. Cocchi. (1986) Oestrous cycle-related LH responsiveness to naloxone: Effect of high oestrogen levels on the activity of opioid receptors. J. Endocr. 108:89-94.

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

Pfaff, D.W. (1980) Estrogens and Brain Function. Springer Verlag. New York.

Pfaff, D.W., and M. Keiner. (1973) Atlas of estradiol-concentrating cells in the central nervous system of the female rat. J. Comp. Neurol. 151:121-158.

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

Pfaff, D.W., and Y. Sakuma. (1979b) Deficit in the lordosis reflex of female rats caused by lesions in the ventromedial nucleus of the hypothalamus. J. Physiol. 288:203-210.

Pfaus, J.G., N. Pendleton, and B.B. Gorzalka. (1986) Dual effect of morphiceptin on lordosis behavior: Possible mediation by different opioid receptor subtypes. Pharmacol. Biochem. Behav. 24:1461-1464.

Pfaus, J.G., and B.B. Gorzalka. (1987a) Selective activation of opioid receptors differentially affects lordosis behavior in female rats. Peptides 8:309-317.

Pfaus, J.G., and B.B. Gorzalka. (1987b) Opioids and sexual behavior. Neurosci. Biobehav. Rev. 11:1-34.

Pfeifle, J.K., and D.A. Edwards. (1983) Midbrain lesions eliminate sexual receptivity but spare sexual motivation in female rats. Physiol. Behav. 31:385-389.

Powers, B., and E.S. Valenstein. (1972) Sexual receptivity: Facilitation by medial preoptic lesions in female rats. Science 175:1003-1005

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

Rainbow, T.C., M.Y. McGinnis, P.G. Davis, and B.S. McEwen. (1982) Application of anisomycin to the lateral ventromedial nucleus of the hypothalamus inhibits the activation of sexual behavior by estradiol and progesterone. Brain Res. 233:417-423.

Rainbow, T.C., B. Parsons, N.J. MacLusky, and B.S. McEwen. (1982) Estradiol receptors in rat hypothalamic and limbic nuclei. J. Neurosci. 2:1439-1445.

Rance, N., P.M. Wise, M. Selmanoff, and C.A. Barraclough. (1981) Catecholamine turnover rates in discrete hypothalamic areas and associated changes in median eminence luteinizing hormone-releasing hormone and serum gonadotropins on proestrous and diestrous day 1. Endocrinology 108:1795-1802.

Renner, K.J., A.W. Smits, D.M. Quadagno and J.C. Hough. (1984) Suppression of sexual behavior and localization of [^{3}H]-puromycin after intracranial injection in the rat. Physiol. Behav. 33:411-414.

Renner, K.J., D. Allen and V.N. Luine (1986) Monoamine levels and turnover in brain: Relationship to priming actions of estrogen. Brain Res. Bull. 16:469-475.

Renner, K.J., L. Krey and V.N. Luine (1987) Effect of progesterone on monoamine turnover in the brain of the estrogen-primed rat. Brain Res. Bull. 19:195-202.

Rodriguez-Sierra, J.F., and G.A. Davis. (1979) Tolerance to the lordosis-facilitating effects of progesterone or methysergide. Neuropharmacology 18:335-339.

Romano, G.J., R.E. Harlan, B.D. Shivers, R.D. Howells, and D.W. Pfaff. (1986) Estrogen increases proenkephalin mRNA levels in the mediobasal hypothalamus of the rat. Society of Neuroscience Annual Meeting, Abstract no. 188.17.

Ronai, A.Z., F.F. Foldes, E.F. Hahn, and J. Fishman. (1977) Orientation of the oxygen atom at C-6 as a determinant of agonistic activity in the oxymorphone series. J. Pharacol. Exp. Therap. 200:496-500.

Roth, R.H., P.M. Salzman and M.C. Nowycky. (1978) Impulse flow and short-term regulation of transmitter biosynthesis in central catecholaminergic neurons. In: Psychopharmacology: A Generation of Progress. Editted by M.A. Lipton, A. DiMascio and K.F. Killam. Raven Press, New York, pp. 185-198.

Rubin, B.S., and R.J. Barfield. (1983) Induction of estrous behavior in ovariectomized rats by sequential replacement of estrogen and progesterone to the ventromedial hypothalamus. Neuroendocrinology 37:218-224.

Sakuma, Y, and T. Akaishi. (1987) Leumorphin, a novel opoiod peptide, promotes lordosis in female rats. Brain Res. 407:401-404.

Saper, C.B., L.W. Swanson, and W.M. Cowan. (1976) The efferent connections of the ventromedial nucleus of the hypothalamus of the rat. J. Comp. Neurol. 169:409-442.

Schechter, P.J., W. Lovenberg, and A. Sjoerdsma. (1972) Dissociation of morphine tolerance and dependence from brain serotonin synthesis rate in mice. Biochem. Pharmacol. 21:751-753.

Shannon, N.J., W.J. Gunnet, and K.E. Moore. (1986) A comparison of biochemical indices of 5-hydroxytryptaminergic neuronal activity following electrical stimulation of the dorsal raphe nucleus. J. Neurochem. 47:958-965.

Siegel, R.A., I. Chowers, N. Conforti, S. Feldman, and J. Weidenfeld. (1982) Effects of naloxone on basal and stress-induced ACTH and corticosterone secretion in the male rat- site and mechanism of action. Brain Res. 249:103-109.

Simerly, R.B., and L.W. Swanson. (1986) The organization of neural inputs to the medial preoptic nucleus of the rat. J. Comp. Neurol. 246:312-342.

Sirinathsinghji, D.J.S. (1984) Modulation of lordosis behavior of female rats by naloxone, beta-endorphin and its antiserum in the mesencephalic central gray: Possible mediation via GnRH. Neuroendocrinology 39:222-230.

Sirinathsinghji, D.J.S. (1986) Regulation of lordosis behavior in the female rat by corticotropin-releasing

factor, beta-endorphin/corticotropin and luteinizing hormone-releasing hormone neuronal systems in the medial preoptic area. Brain Res. 375:49-56.

Snavely, M.D., M.G. Ziegler and P.A. Insel. (1985) Subtype-selective down-regulation of rat renal cortical alpha- and beta-adrenergic receptors by catecholamines. Endocrinology 117:2182-2188.

Sourkes, T.L. (1966) DOPA Decarboxylase: Substrates, coenzyme, inhibitors. Pharmacol. Rev. 18:53.

Spampinato, U., E. Esposito, S. Romandini, and R. Samanin. (1985) Changes of serotonin and dopamine metabolism in various forebrain areas of rats injected with morphine either systemically or in the raphe nuclei dorsalis or medianus. Brain Res. 328:89-95.

Spector, S., R. Gordon, A. Sjoerdsma, and S. Udenfriend. (1967) End-product inhibition of tyrosine hydroxylase as a possible mechanism for regulation of norepinephrine synthesis. Mol. Pharmacol. 3:549-555.

Tagliamonte, A., P. Tagliamonte, J. Perez-Cruet, S. Stern, and J.L. Gessa. (1971) The effect of psychotropic drugs on tryptophan concentration in the rat brain. J. Pharmacol. Exp. Therap. 177:475-480.

Tempel, A., E.L. Gardner, and R.S. Zukin. (1984) Visualization of opiate receptor upregulation by light microscopy autoradiography. Proc. Natl. Acad. Sci. 81:3893-3897.

Tempel, A., S.M. Crain, E.R. Peterson, E.J. Simon, and R.S. Zukin. (1986) Antagonist-induced opiate receptor upregulation in cultures of fetal mouse spinal cord-ganglion explants. Dev. Brain Res. 25:287-291.

Tempel, A.,and R.S. Zukin. (1987) Neuroanatomical pattern of the mu, delta, and kappa opioid receptors of rat brain as determined by in vitro autoradiography. Proc. Natl. Acad. Sci. 84:4308-4312.

Tennent, B.J., E.R. Smith and J.M. Davidson. (1982) Effects of progesterone implants in the habenula and midbrain on proceptive and receptive behavior in the female rat. Hormones Behav. 16:352-363.

Thornton, J.E., B. Nock, B.S. McEwen and H.H. Feder. (1986) Noradrenergic modulation of hypothalamic progestin receptors

in female guinea pigs is specific to the ventromedial nucleus. Brain Res. 377:155-159.

Tozer, T.N., N.H. Neff, and B.B. Brodie. (1966) Application of steady state kinetics to the synthesis rate and turnover time of serotonin in the brain of normal and reserpine-treated rats. J. Pharmacol. Exp. Therap. 153:177-182.

Turner, C.D., and J.T. Bagnara. (1976) General Endocrinology. W.B. Saunders, Philadelphia.

Udenfriend, S. (1966) Tyrosine hydroxylase. Pharmacol. Rev. 18:43-51.

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

Van Loon, G.R., A. Shum, and M.J. Sole. (1981) Decreased brain serotonin turnover after short-term (two hour) adrenalectomy in rats: A comparison of four turnover methods. Endocrinology 108:1392-1402.

Van Vugt, D.A., P.W. Sylvester, C.F. Aylsworth, and J. Meites. (1982) Counteraction of gonadal steroid inhibition of luteinizing hormone release by naloxone. Neuroendocrinology 34:274-278.

Vincent, R., T. Hokfelt, I. Christensson and L. Terenius. (1982) Dynorphin-immunoreactive neurons in the central nervous system of the rat. Neurosci. Lett. 33:185-190.

Walter, J.R., and R.H. Roth. (1976) Dopaminergic neurons-alteration in the sensitivity of tyrosine hydroxylase to inhibition by endogenous dopamine after cessation of impulse flow. Biochem. Pharm. 25:649-654.

Walters, M.R. (1985) Steroid hormone receptors and the nucleus. Endocrine Rev. 6:512-543.

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

Warembourg, M. (1985) Steroid receptors in the brain: Topography and some functional implications. Neurochem. Int. 7:941-952.

Watson, R.E., Jr., G.E. Hoffman, and S.J. Wiegand. (1986) Sexually dimorphic opioid distribution in the preoptic area: Manipulation by gonadal steroids. Brain Research 398:157-163.

Weiner, N. (1974) A critical assessment of methods for the determination of monoamine synthesis turnover rates in vivo. In: Neuropharmacology of Monoamines and their Regulatory Enzymes, edited by E. Usdin. Raven Press, New York, pp. 143-159.

Werling, L.L., S.R. Brown, and B.M. Cox. (1987) Opioid receptor regulation of thej release of norepinephrine in brain. Neuropharmacology 26:987-996.

Wiesner, J.B., and R.L. Moss. (1984) Beta-endorphin suppression of lordosis behavior in female rats; Lack of effect of peripherally-administered naloxone. Life Sciences 34:1455-1462.

Wiesner, J.B., and R.L. Moss. (1986a) Suppression of receptive and proceptive behavior in ovariectomized estrogen-progesterone-primed rats by intraventricular beta-endorphin: Studies of behavioral specificity. Neuroendocrinology 43:43:1235-1239.

Wiesner, J.B., and R.L. Moss. (1986b) Behavioral specificity of beta-endorphin suppression of sexual behaivior: Differential receptor antagonism. Pharmacol. Biochem. Behav. 24:1235-1239.

Wiesner, J.B., J.I. Koenig, L. Krulich, and R.L. Moss. (1984) Site of action for beta-endorphin-induced changes in plasma Luteinizing Hormone and prolactin in the ovariectomized rat. Life Sciences 34:1463-1473.

Wilcox, J.N., and J.L. Roberts. (1985) Estrogen decreases rat hypothalamic proopiomelanocortin messenger ribonucleic acid levels. Endocrinology 117:2392-2396.

Wilkinson, M., R. Bhanot, D.A. Wilkinson, and R.J. Brawer. (1983) Prolonged estrogen treatment induces changes in opiate, benzodiazepine and beta-adrenergic binding sites in female rat hypothalamus. Brain Res. Bull. 11:279-281.

Yamano, M., S. Inagaki, S. Kito, and M. Tohyama. (1985) An enkephalinergic projection from the hypothalamic paraventricular nucleus to the hypothalamic ventromedial nucleus of the rat: An experimental immunohistochemical study. Brain Research 331:25-33.

Yamano, M., S. Inagaki, S. Kito, T. Matsuzaki, Y. Shinohara, and M. Tohyama. (1986) Enkephalinergic projection from the ventromedial hypothalamic nucleus to the midbrain central gray matter in the rat: An immunohistochemical analysis. Brain Research 398:337-346.

Zar, J.H. (1974) Biostatistical Analysis. Prentice-Hall, Englewood Cliffs, NJ.

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

Zukin, R.S., and A. Tempel. (1986) Neurochemical correlates of opiate receptor regulation. Biochem. Pharmacol. 35:1623-1627.