PROGESTERONE'S MODULATION OF SEROTONIN 1A RECEPTOR FUNCTIONING IN THE OVARIECTOMIZED ESTROGEN-TREATED RAT

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DEDICATION PAGE

To Susan: for her support, friendship, and selfless endurance throughout

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ABSTRACT

PROGESTERONE'S MODULATION OF SEROTONIN 1A RECEPTOR FUNCTIONING IN THE OVARIECTOMIZED ESTROGEN-TREATED RAT

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The focus of this study was to determine if progesterone regulates serotonergic control of lordosis behavior in estradiol benzoate (EB)-primed ovariectomized (OVX) rats. It was predicted that progesterone would decrease the lordosis-inhibiting effects of the 5-HT_{1A} receptor agonist, 8-OH-DPAT. The drug, given systemically, was indeed less effective at inhibiting the lordosis reflex in the EP (rats primed with 10 μg EB and 500 μg progesterone 48 hr later) rats when compared to the EO (rats primed with 10 μg EB and oil 48 hr later) rats. Furthermore, when the OVX rats were preprimed with EB (10 μg EB, 7 days prior to hormone priming), a greater attenuation of the lordosis-inhibiting effect of 8-OH-DPAT was present in the EP preprimed rats compared to the EO preprimed rats. It was hypothesized that the reduction in potency of 8-OH-DPAT to inhibit lordosis behavior after progesterone priming was related to one of these three mechanisms: (1) decreasing extracellular 5-HT, (2) decreasing the effectiveness of 5-HT_{1A} receptors in the ventromedial nucleus of the hypothalamus (VMN) or (3) increasing the ability of 5-$HT_{2A/2C}$ receptors to enhance lordosis behavior, masking the effects of the 5-HT_{1A} receptor agonists. Microdialysis of OVX rats was used to determine that progesterone (500 μg) after EB (2.5 or 25 μg) reduced extracellular 5-HT. 5-HT levels were reduced to the limits of detectability in the medial basal

hypothalamus (MBH), while rats given either dose of EB without progesterone had extracellular 5-HT levels similar to vehicle treatment. When 8-OH-DPAT was infused directly into the VMN, there was no difference in lordosis behavior of EP or EO rats, and no apparent effects of progesterone to decrease the effectiveness of 5-HT_{1A} receptors in the VMN were observed. Lastly, the effects of progesterone on the $5\text{-HT}_{2A/2C}$ receptor-mediated behaviors, wet dog shakes and back muscle contractions, were examined. Progesterone priming in EB-primed OVX rats produced no differences in either behavior compared to EB-primed OVX rats. It is likely that the progesterone-induced decrease in potency of 8-OH-DPAT to inhibit lordosis behavior is related to decreased endogenous levels of extracellular 5-HT.

LIST OF ABREVIATIONS

Word	Abbreviation
(±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane	DOI
(±)-8-hydroxy-2-(di-*n*-propylamino)tetralin	8-OH-DPAT
Back Muscle Contractions	BMC
Cyclic AMP	cAMP
Dorsal Raphe Nucleus	DR
Effective Dose 50% maximal	ED_{50}
Estradiol Benzoate	EB
Estrogen Receptor	ER
Inositol-1,4,5-triphosphate	IP_3
Intraperitoneal	i.p.
Lordosis to Mount Ratio	L/M
Medial Basal Hypothalamus	MBH
Medial Reticular Formation	MRF
Midbrain Central Grey	MCG
Ovariectomized	OVX
Preprimed with Estradiol Benzoate and Primed with Estradiol Benzoate and Oil	EEO
Preprimed with Estradiol Benzoate and Primed with Estradiol Benzoate and Progesterone	EEP
Primed with Estradiol Benzoate and Oil	EO
Primed with Estradiol Benzoate and Progesterone	EP
Progesterone Receptor	PR
Serotonin transporter	SERT
Serotonin	5-HT
Sesame Seed Oil	oil
Subcutaneous	s.c.
Ventromedial Nucleus of the Hypothalamus	VMN
Wet Dog Shakes	WDS

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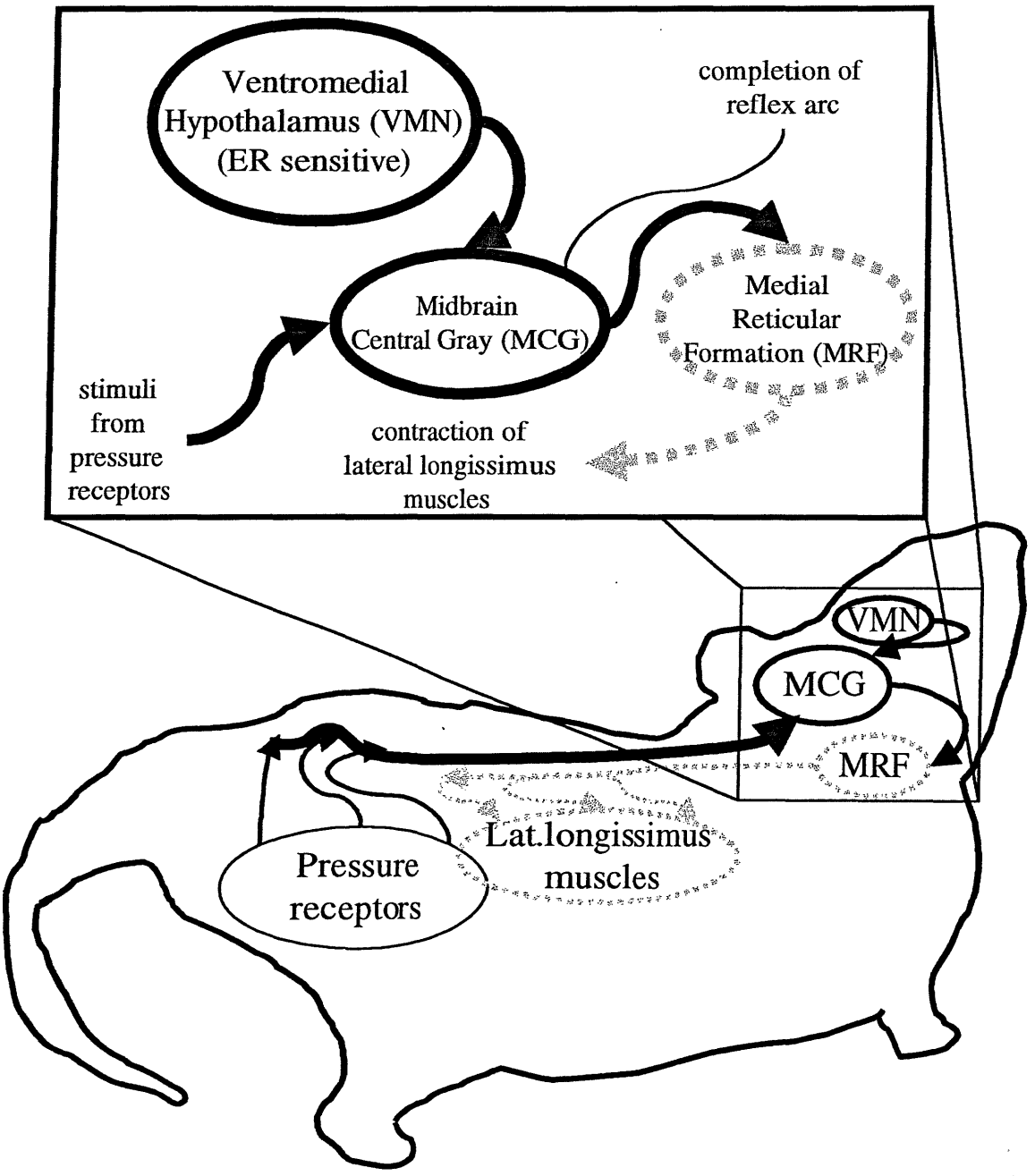
CHAPTER I

1. Introduction

The goal of neuroscience, ultimately, is to understand the physiological basis of behaviors. Behaviors are generally described as actions or reactions of an organism to stimuli. The most rudimentary behavior is a simple reflex, where a stimulus produces an involuntary response. The simplest somatic motor reflex occurs at the level of the spinal cord and involves a two-neuron circuit. An afferent neuron, carrying the sensory information, synapses onto an efferent neuron in the ventral horn of the spinal cord. The axon of the efferent neuron leaves the spinal cord to innervate skeletal muscles and mediate the response (typically a muscle contraction) to the sensory input. Not all reflexes are simple and not all reflexes occur at the level of the spinal cord.

The lordosis reflex is an example of a supraspinal reflex. It represents a complex behavior and is under both neural and hormonal control. The reflex is part of the female mating behavior in the rat. It involves tactile input from a male rat and results in an arching of the back of the female rat. This process is necessary for successful copulation to occur. A simplified model of the circuitry of the reflex is outlined in Figure 1 (for a more extensive review see [47]). The reflex is initiated by stimulation of pressure receptors located in the flank, posterior rump or perineum of the female rat. A signal from these receptors is carried via the pudendal nerve to the dorsal roots of the spinal cord (T_{11} through S_3), where it synapses onto second order neurons. The second order neurons carry the ascending information to the midbrain via the anterolateral

Figure 1: A simplified model of the neural pathway of the lordosis reflex.
In this diagram is shown a simplified model for the neuronal circuit involved in the lordosis reflex. Solid lines represent the path of the afferent signal and dashed lines represent the part of the efferent signal. Estrogen dependent input from the VMN completes the reflex arc in the midbrain.



column. Information carried by these neurons ultimately reaches the midbrain where axons terminate at the lateral borders of the midbrain central gray. The midbrain central gray also receives hypothalamic inputs. Of special importance to the control of the lordosis reflex is input from the medial basal hypothalamus (MBH). Estrogen increases activation of MBH neurons, some of which innervate the midbrain central gray. Such estrogen-dependent activation is thought to lead to facilitation of lordosis behavior [47]. Lordosis-relevant projections, from the midbrain central gray, travel to the medullary reticular formation from which axons are carried by the lateral vestibulospinal and reticulospinal tracks. These axons synapse at spinal motor neurons that innervate the muscles of the lateral longissimus dorsi and the multifidus system. Stimulation of these muscles leads to the arching of the back, or lordosis.

Female gonadal hormones, especially estrogen and progesterone, control the lordosis reflex in response to tactile stimuli from the male. In an intact, naturally cycling female rat, the lordosis reflex will only occur during the night of proestrous [9]. It is during this phase that the female rat is said to be sexually receptive. Prior to this period of sexual receptivity, levels of estrogen gradually rise to reach peak levels on the afternoon of proestrous [9]. About the same time that estrogen levels reach their peak, progesterone levels increase rapidly and peak near the onset of sexual receptivity [9]. If a rat is ovariectomized (OVX) prior to the progesterone surge (approximately 3 hr prior to the expected onset of receptivity), lordosis behavior does not occur. However, if the ovariectomy is followed by an injection of progesterone, lordosis behavior is similar to that of an intact proestrous female [50]. If the rat is OVX 17 hr prior to the expected onset of receptivity so that the estrogen peak is eliminated, lordosis does not occur.

Inclusion of progesterone in this OVX rat, without estrogen priming, still does not induce the lordosis behavior. However, if ovariectomy is performed 11 hr prior to the proestrous phase and primed with progesterone the rat will exhibit lordosis behavior [43]. These findings are consistent with the generally accepted view that, in the naturally cycling female rat, at least 16 hr of estrogen priming, followed by the effect of progesterone, are required to elicit lordosis behavior.

The ability of estrogen and progesterone to influence lordosis behavior in OVX rats are well documented. Although OVX rats treated only with estrogen can show lordosis behavior in the absence of progesterone, supraphysiological doses of estrogen are required [10,12,48,54]. With the addition of progesterone priming, the dose of estrogen required to induce lordosis is decreased [3,7,25]. Moreover, the full repertoire of behavior shown by a sexually receptive intact female rat can only be initiated in an OVX rat by sequential estrogen and progesterone priming [3].

The mechanisms whereby estrogen and progesterone influence the lordosis reflex have been the subject of considerable research. Estrogen's action appears to require the binding of estrogen to the estrogen receptor (ER). The ER, typical of many steroid receptors, functions as a transcription factor, regulating transcription of genes with appropriate estrogen response elements. For example, early work established the importance of the ER. Meisel and co-workers [41] reported that ER antagonists (anti-estrogens) given to an OVX rat 24 hr prior to estrogen or estrogen and progesterone priming prevented the hormonal elicitation of lordosis behavior. Furthermore, this antagonism was present when the anti-estrogens were placed in the MBH, specifically in the ventromedial nucleus of the hypothalamus (VMN), but not when placed in other

hypothalamic areas or in other portions of the CNS. Estrogen's induction of the lordosis reflex could also be attenuated when agents, such as actinomycin-D, ethidium bromide and netropsin, that disrupt the nuclear binding of steroid-receptor complexes to the DNA, were infused into the VMN 1 hr prior to estrogen treatment [14]. Protein synthesis inhibitors after estrogen also attenuate the induction of lordosis behavior [40,53,55]. In contrast, agents that increase ER binding increase lordosis behavior [33,38] and the products of genes that are activated by the ER-complex facilitate lordosis behavior [19]. It is now known that two ER's, the ER alpha and the ER beta, exist. Most recently, it was reported that ER alpha knockout mice do not show sexual receptivity after estrogen priming, while ER beta knockout mice do show lordosis behavior after hormone replacement ([44,45] respectively). Thus, ER alpha is currently thought to be required for estrogen's induction of lordosis behavior.

One of the gene products activated by estrogen is the progesterone receptor (PR). Progesterone's facilitation of lordosis in an estrogen-primed OVX rat appears to depend on progesterone binding to the intracellular PR [4-6] and, the PR antagonist, RU486, attenuates progesterone-facilitated lordosis [13,24]. However, there is evidence that progesterone may also facilitate lordosis by nongenomic mechanisms that are independent of the PR [11,20,21]. For example, progesterone has been demonstrated to increase lordosis in estrogen-primed PR knockout mice [21]. One or more of progesterone's metabolites may mediate these putative nongenomic mechanisms of progesterone-facilitated lordosis. These metabolites have been reported to enhance $GABA_A$ receptor activation [52], which increases Cl^- conductance and hyperpolarizes neurons, and $GABA_A$ receptor agonists can facilitate lordosis behavior [37]. Thus,

progesterone-facilitated lordosis may include both genomic and non-genomic effects of the hormone.

Although estrogen is essential for elicitation of lordosis behavior, progesterone is not the only additional regulator of lordosis behavior. Neurotransmitters can also regulate this behavior. The serotonergic system is an example of a neurotransmitter system that is involved with the regulation of lordosis. There are at least seven main families of serotonin (5-HT) receptors [18]. Of these, two families have been extensively studied in the modulation of lordosis; these are the 5-HT_1 and the 5-HT_2 receptor families. 5-HT_1 receptors can be negatively coupled to adenylyl cyclase and decrease cAMP, while the 5-HT_2 family is positively coupled to phospholipase C and increases phosphatidylinositol turnover and increases IP_3 [18]. As a general finding, compounds that decrease cyclic AMP (cAMP) in the VMN inhibit the behavior, while compounds that increase cAMP or inositol-1,4,5-triphosphate (IP_3) increase lordosis behavior [30].

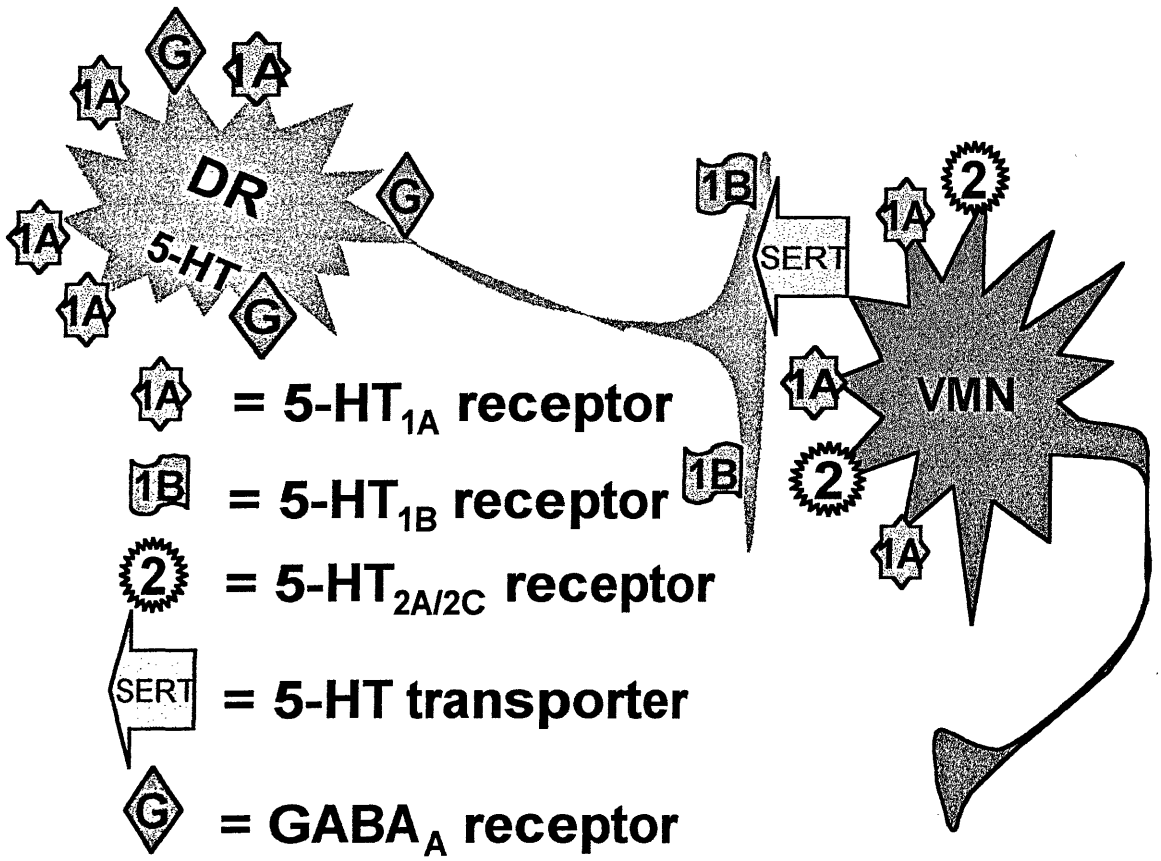
5-HT is generally regarded as being inhibitory to the lordosis reflex and removal of hypothalamic 5-HT neurons by the 5-HT neurotoxin, 5,7-dihydroxytryptamine, enhances estrogen's facilitation of the lordosis reflex [17,34]. Moreover, both systemic injections of and intracranial infusions of the 5-HT_{1A} receptor agonist, (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), have been reported to reduce lordosis behavior in sexually receptive, proestrous females and in OVX rats primed with estrogen or with estrogen and progesterone [1,42,61,63]. 5-HT's inhibition of lordosis is now thought to be mediated in part by activation of 5-HT_{1A} receptors. Furthermore, the ability of 5-HT_{1A} receptor agonists to inhibit the lordosis reflex in rats after removal

of serotonergic terminals by the use of 5,7-dihydroxytryptamine has led to the suggestion that 5-HT's inhibition of lordosis behavior occurs via activation of postsynaptic 5-HT_{1A} receptors [2]. An effective site for this postsynaptic inhibition of the lordosis reflex by 5-HT_{1A} receptor agonists is the VMN [59], which receives endogenous serotonergic inputs from the dorsal raphe nucleus (see Figure 2).

Although 5-HT's role in modulation of the lordosis reflex is predominantly inhibitory, it has been suggested that 5-HT may also have facilitatory influences on the reflex via 5-$HT_{2A/2C}$ receptor activation [42]. This suggestion is mainly based on reports that 5-$HT_{2A/2C}$ receptor antagonists can inhibit lordosis of intact and hormone-primed OVX rats [42,62,64], and that 5-$HT_{2A/2C}$ receptor agonists can facilitate the reflex in OVX rats sub-optimally primed with estrogen and progesterone [23,65,66]. There are also reports that 5-$HT_{2A/2C}$ receptor agonists can attenuate the suppression of neuronal firing and the inhibition of the lordosis reflex after treatment with 5-HT_{1A} receptor agonists [31,35]. Thus, 5-HT has been suggested to have a dual role in the modulation of the lordosis reflex.

Many investigators have suggested that estrogen, progesterone, or both may modify the serotonergic system. Lakoski [32] reported that estrogen decreased the ability of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, to suppress 5-HT neuronal firing, and suggested that estrogen desensitized or downregulated the 5-HT_{1A} receptor. Jackson and Uphouse [27] reported that estrogen dose-dependently attenuated the lordosis-inhibiting effects of 8-OH-DPAT in OVX rats also primed with progesterone. It has also been reported that prepriming an OVX rat with estrogen (an injection of estrogen 4 to 12 days prior to regular hormone priming with estrogen and progesterone)

Figure 2: Serotonergic inputs into the VMN



Serotonergic inputs from the DR to the VMN. Somatodendritic 5-HT_{1A} receptors located in the DR act as autoreceptors to decrease cell firing, as do $GABA_A$ receptors, also located on 5-HT neurons. The 5-HT_{1B} receptor acts as the terminal autoreceptor and decreases the amount of 5-HT released per action potential. Progesterone can enhance activation of the $GABA_A$ receptors via its metabolites, and progesterone also increases binding to 5-HT_{1B} receptors in the VMN. On the postsynaptic neurons, activation of the 5-HT_{1A} receptors inhibits lordosis, and activation of the 5-HT_2 receptors can facilitate lordosis and/or override the inhibitory effects of 5-HT_{1A} receptor agonists.

decreases the potency of systemic injection of 8-OH-DPAT to inhibit the lordosis reflex [28,60]. Trevino and co-workers [57] reported that prepriming with estrogen led to a decrease in the efficacy of 8-OH-DPAT to inhibit lordosis when the 8-OH-DPAT was infused into the VMN and suggested a decrease in the effectiveness of 5-HT_{1A} receptors in the VMN. Thus, estrogen's regulation of lordosis behavior may be, in part, linked to its regulation of the serotonergic system.

There is also evidence that progesterone regulates the serotonergic system. Progesterone after estrogen priming appears to decrease 5-HT. Gereau and co-workers [22] reported that progesterone following estrogen led to a decrease in tissue levels of 5-HT, and Farmer and co-workers [15] reported that progesterone led to a decrease in extracellular 5-HT of anesthetized estrogen-primed OVX rats. Although both of these studies are consistent, it is not known whether this trend would occur in a freely moving rat.

Progesterone has also been reported to modulate 5-HT receptors. Frankfurt and co-workers [17] reported an increase in 5-HT_{1B} receptor binding in the VMN of OVX rats primed with estrogen and progesterone compared to rats primed with estrogen alone. The 5-HT_{1B} receptor acts as a terminal autoreceptor on 5-HT neurons [18], and its activation decreases the amount of 5-HT released per action potential. Thus, progesterone may decrease extracellular 5-HT by enhancing 5-HT_{1B} terminal autoreceptors. In this same study, it was also reported that progesterone did not alter 5-HT_{1A} binding. If progesterone decreases extracellular 5-HT and if activation of postsynaptic 5-HT_{1A} receptors decreases lordosis behavior, progesterone may facilitate lordosis, in part, by decreasing 5-HT's inhibitory influence on the behavior. Moreover,

progesterone would be expected to attenuate 5-HT's inhibition of the behavior. However, very little is known regarding progesterone's effect on the serotonergic control of the lordosis reflex.

The current study was designed to determine the role of progesterone on 5-HT and 5-HT-mediated changes in lordosis behavior. It was hypothesized that progesterone after estrogen priming would decreases the lordosis-inhibiting effect of the 5-HT_{1A} receptor agonist, 8-OH-DPAT. There are three possible mechanisms whereby progesterone may do this: (1) by decreasing the amount of 5-HT available to activate 5-HT_{1A} receptors; (2) by decreasing the effectiveness of the 5-HT_{1A} receptor to inhibit the lordosis reflex or; (3) by increasing the ability of 5-$HT_{2A/2C}$ receptors to enhance the lordosis reflex or attenuate the effects of the 5-HT_{1A} receptor agonists.

One mechanism by which progesterone may decrease 8-OH-DPAT's ability to inhibit the lordosis reflex is by removing inhibition of lordosis behavior by decreasing extracellular 5-HT in the VMN. Progesterone's influence on extracellular 5-HT, sampled by microdialysis, was assessed in freely moving OVX rats to evaluate this possibility. Microdialysis was performed in the MBH since the VMN is contained within this brain region. It is within the VMN that a decrease in extracellular 5-HT would be expected to reduce inhibition of lordosis behavior following a 5-HT_{1A} receptor agonist.

If progesterone reduces extracellular 5-HT in the VMN, less endogenous 5-HT would be available for activation of postsynaptic 5-HT_{1A} receptors, and a greater concentration of 8-OH-DPAT in the VMN would be required to inhibit lordosis behavior. Therefore, a progesterone-induced decrease in extracellular 5-HT in the VMN

should decrease the effectiveness of systemic injections of 8-OH-DPAT. Administered systemically, 8-OH-DPAT will activate both pre and postsynaptic 5-HT_{1A} receptors. Activation of presynaptic 5-HT_{1A} receptors in the dorsal raphe (DR) leads to a decrease in 5-HT neuronal firing and, subsequently, a decrease in 5-HT release [18]. Since a major source of serotonergic input into the VMN is from the DR [18], the progesterone-induced decrease in extracellular 5-HT in the VMN may be further decreased by systemic treatment of 8-OH-DPAT. Thus, a larger dose of 8-OH-DPAT would be required to activate enough postsynaptic VMN 5-HT_{1A} receptors to inhibit the lordosis reflex in estrogen and progesterone-primed rats compared to rats primed only with estrogen.

A second mechanism by which progesterone may decrease the ability of 8-OH-DPAT to inhibit the lordosis reflex is by decreasing the effectiveness of the 5-HT_{1A} receptor. Trevino et al. [57] reported that prior treatment with estrogen decreased the efficacy of 8-OH-DPAT when infused into the VMN of OVX rats primed with estrogen and progesterone. Since estrogen induces progesterone receptors (PR) [46,49], prior treatment with estrogen may have enhanced progesterone's effects; thus, the decreased effectiveness of the postsynaptic 5-HT_{1A} receptor after estrogen pretreatment could be related to progesterone treatment. If the estrogen-induced decrease in efficacy of 8-OH-DPAT is mediated through the effects of progesterone, then progesterone priming could decrease the efficacy of VMN infusions of 8-OH-DPAT in OVX rats primed with a single estrogen treatment.

Finally, progesterone may decrease the effect of 5-HT_{1A} receptor agonists by enhancing mechanisms involved in facilitating lordosis behavior. Activation of

5-$HT_{2A/2C}$ receptors facilitates the lordosis reflex in rats [23,65], and Wilson and Hunter [64] suggested that progesterone facilitates the lordosis reflex by increasing the function of 5-$HT_{2A/2C}$ receptors. Since it has been established that 5-$HT_{2A/2C}$ receptor agonists can attenuate the effects of 5-HT_{1A} receptor agonists, it is possible that progesterone may attenuate 8-OH-DPAT-induced inhibition of the lordosis reflex by enhancing 5-$HT_{2A/2C}$ receptor functioning. Since rats that receive both estrogen and progesterone are likely to show high levels of lordosis behavior, it would be difficult to assess differential effects of estrogen or estrogen plus progesterone on 5-$HT_{2A/2C}$ receptor-facilitated lordosis behavior. However, assessment of progesterone's influence on 5-$HT_{2A/2C}$ receptors is possible by utilizing a different behavioral paradigm. Two behaviors that are increased by 5-$HT_{2A/2C}$ receptor agonists, such as (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), are wet dog shakes (WDS) and back muscle contractions (BMC). Assessment of these behaviors in OVX rats primed with estrogen ± progesterone allows for examination of the effects of progesterone on 5-$HT_{2A/2C}$ receptor functioning. Furthermore, differentiation between the effects modulated by the 5-HT_{2A} receptor and the 5-HT_{2C} receptor may be possible since co-injection with DOI and a selective 5-HT_{2A} receptor antagonist prevents WDS, while co-injection with a selective 5-HT_{2C} receptor antagonist reduces BMC. Consequently, it has been suggested that WDS are mediated by 5-HT_{2A} receptors and BMC are mediated by 5-HT_{2C} receptors [51,56]. If progesterone does increase activation of either the 5-HT_{2A} or 5-HT_{2C} receptor or both, then OVX rats primed with estrogen and progesterone should display an increase in one or both of these behaviors compared to OVX estrogen-primed rats.

The current studies are designed to examine the potential role of progesterone in the attenuation of 8-OH-DPAT-induced inhibition of the lordosis reflex. Estrogen-primed OVX rats were used to complete the following specific aims:

1. To determine if progesterone decreases extracellular 5-HT in the MBH,
2. To determine if progesterone decreases the effectiveness of 8-OH-DPAT's inhibition of the lordosis reflex,
3. To determine if progesterone increases 5-$HT_{2A/2C}$ receptor-mediated behaviors.

CHAPTER II

2. Materials and methods

2.1. Materials

Microdialysis probes and guides were purchased from CMA (Acton, MA). HPLC columns and related materials were purchased from BAS (Lafayette, IN). Suture materials were obtained from Butler Co. (Arlington, TX) and methoxyflurane (Metofane®) was purchased from Pitman Moore (Mundelein, IL). Ringer's solution was purchased from Baxter (Deerfield, IL). Intracranial cannulae were obtained from Plastic Products, Inc. (Roanoke, VA) and dental acrylic was obtained from Reliance Dental Mfg. Co. (Worth, IL). Estradiol benzoate (EB), progesterone and sesame seed oil (oil) were purchased from Fisher Scientific (Houston, TX). (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) were purchased from Research Biochemicals Inc. (Natick, MA). All other supplies came from Fisher Scientific (Houston, TX).

2.2. General methods

Female (CDF-344) rats, purchased as adults or bred in the TWU animal facility from stock obtained from Sasco Laboratories (Wilmington, MA), were weaned at 25 days of age and housed three or four per cage in polycarbonate shoebox cages with food and water available *ad lib.* The housing rooms were maintained at 22^0 C and 55% humidity under a reversed 12-12 hr light-dark cycle with lights off at 12 noon.

For microdialysis experiments, female rats weighing between 140 and 170 grams were anesthetized with Metofane® and unilaterally implanted with a CMA 12

microdialysis probe guide with dummy probe. The tip of the probe was directed stereotactically toward the VMN [atlas coordinates from Konig and Klippel AP 4.38; DV 7.8; ML 0.4] to allow for microdialysis of the MBH [29]. For intracranial experiments, female rats weighing between 140 and 170 grams were anesthetized with Metofane® and implanted bilaterally with 22-gauge stainless steel guide cannulae advanced stereotactically toward the VMN [atlas coordinates from Konig and Klippel AP 4.38; DV - 7.8; ML ± 0.4] [29].

All other experiments used OVX rats without cannulae or microdialysis probes. Rats were bilaterally OVX while anesthetized with Metofane®. Ovariectomy of non-implant rats was performed on 60 - 110 day old rats. Rats used in the infusion studies were OVX 2 weeks after the implant surgery and rats used in the microdialysis experiment were OVX at the time of the implant surgery.

Hormonal priming began 1 - 2 weeks after ovariectomy and all hormone injections were administered between 9 and 10 am. Hormones, dissolved in sesame seed oil, were injected subcutaneously (s.c.) in a volume of 0.1 ml per rat. Both 8-OH-DPAT and DOI were dissolved in saline and administered according to the specific requirements of each experiment.

For sexual behavioral studies, at least 1 hr prior to lights off on the day of sexual behavior testing, rats were moved, in their home cage, into the testing room where the males were housed. Testing for sexual behavior, as previously described [61], was initiated within 1-3 hours after colony lights off, and 4 - 6 hr after the progesterone or oil injection. Visibility was aided by red lighting. Females were placed in the home cage of sexually experienced males for the pretest. Sexual receptivity was monitored for

10 mounts, representing the pretest, and only rats with a pretest lordosis to mount (L/M) ratio of 0.7 or higher were included in the remaining procedures. After the pretest, the females were removed, administered the appropriate treatment and returned to the male's cage. Sexual behavior continued to be monitored for 30 consecutive min thereafter. Sexual receptivity was quantified as the lordosis to mount (L/M) ratio (e.g., number of lordosis responses by the female divided by the number of mounts by the male) as previously described [59].

Data were evaluated by either a two-way or three-way repeated measures ANOVA. Differences between hormone treatment groups within time intervals were evaluated with Tukey's test. For the behavioral experiments, comparisons within groups across time after treatment were made with Dunnett's test to the pretest interval. The statistical reference was Zar [67], and an alpha level of 0.05 was required for rejection of the null hypothesis.

2.3. Specific procedures

2.3.1. Experiment 1

Experiment 1 was designed to determine the effects of EB and progesterone on extracellular 5-HT. Twenty-seven rats were implanted with microdialysis probe guides and were OVX. Two weeks after surgeries, rats were injected with oil, with 2.5 μg or 25 μg EB, or with either dose of EB followed 48 hr later with 500 μg progesterone. Injections occurred between 9 and 10 am (2 to 3 hr prior to onset of the dark cycle).

Prior to microdialysis, each animal was adapted to the containment system (BAS, Lafayette, IN) for a minimum of 3 days. At 8:00 am on the day of the experiment, a CMA 12 microdialysis probe (2 mm in length) replaced the animal's

dummy probe and was perfused continuously with Ringer's solution at a flow rate of 1 µl/min. After a 2-hr stabilization period, microdialysate samples, collected at consecutive 30-min intervals, were evaluated for 5-HT content. At the conclusion of the experiment, histological evaluation and reference to Konig and Klippel [29] confirmed probe location. Only rats with correct probe location were included for statistical analysis.

HPLC determinations of 5-HT were made by electrochemical detection with a PM 80 pump (BAS, Lafayette, IN), a 9125 Rheodyne injector (10 µl loop), a MF-8949 microbore column, a Unijet electrode (6 mm) and a LC 4C Amperometric controller (BAS, Lafayette, IN). The mobile phase consisted of 0.09 M citric acid, 0.07 M Na_2HPO_4, 0.10 mM EDTA, 2.62 nM sodium octyl sulfate and 13% methanol, adjusted to a pH of 3.62. NaCl (10 mM) was included in the buffer for operation of the Unijet detector. Elution was performed at a flow rate of 60 µl/min and the potential for electrochemical detection was 650 mV. Quantitative determination was determined by comparison with appropriate external standards. Data are reported as picograms per 10 µl of microdialysate sample.

2.3.2. Experiment 2

The second experiment was designed to determine if the addition of progesterone to an EB-primed rat would decrease the lordosis-inhibiting effect of systemic injections of 8-OH-DPAT. To accomplish this, eighty-four OVX rats were injected, two weeks after surgery, with 10 µg EB followed 48 hr later with either 500 µg progesterone (EP) or 0.1 ml of oil (EO). Four to six hr after the last injection rats were pretested for sexual behavior. Females were then injected, i.p., with saline, 0.05 mg/kg, 0.075 mg/kg, or

0.1 mg/kg 8-OH-DPAT, and behavioral testing continued for 30 consecutive min.

2.3.3. Experiment 3

The third experiment was designed to determine the role of progesterone in the attenuation of the lordosis-inhibiting effects of 8-OH-DPAT by prepriming with EB. Sixty-two OVX rats were injected with 10 μg EB (prepriming) or oil 7 days after surgery. Seven days after the initial injection all rats were injected with 10 μg EB followed 48 hr later by either an injection of 500 μg progesterone or oil. This hormone regimen produced 4 groups of rats: rats receiving an oil injection followed by EB ± progesterone (OEP, OEO) and rats preprimed with EB followed by EB ± progesterone (EEP, EEO). Four to six hr after the last injection, females were pretested for sexual behavior (as described above). Females were then injected i.p. with either 0.15 mg/kg or 0.5 mg/kg 8-OH-DPAT and returned to the males cage. Sexual behavior was recorded continuously for the next 30 min.

2.3.4. Experiment 4

The fourth experiment was designed to determine if the addition of progesterone to an EB-primed rat would decrease the lordosis-inhibiting effect of VMN infusions with 8-OH-DPAT. Forty-four OVX rats, implanted bilaterally with VMN guide cannulae, were injected, two weeks after ovariectomy, with 10 μg EB followed 48 hr later with 500 μg progesterone (EP) or 0.1 ml of oil (EO). Four to six hr after the last injection, rats were pretested for sexual behavior. Females were then removed from the male's home cage, placed in a BAS containment system where the dummy stylets were replaced by 28 gauge cannulae. Saline, 50 ng, or 100 ng 8-OH-DPAT in a volume of 0.5 μl were infused bilaterally by a CMA 170 infusion pump (Acton, MA). After the

infusion was complete, the cannulae were removed from the guide cannulae and the female was again placed in the home cage of the male, and sexual behavior was continually monitored for 30 min.

2.3.5. Experiment 5

Experiment 5 was designed to determine if progesterone increased DOI-mediated behaviors. Twenty-four OVX rats were used. Two weeks after surgery, rats were injected with 10 μg EB followed 48 hr later by an injection with either 500 μg P or oil. Four to six hr after the last injection, rats were injected with 1.5 or 2.5 mg/kg DOI, i.p., and were placed in a paper-lined shoebox polycarbonate cage, where they were videotaped for 30 min after injection. The videotapes were reviewed for purposes of monitoring the 5-$HT_{2A/2C}$ receptor-mediated behaviors, WDS and BMC. Each instance of a WDS, shaking of the head back and forth, or of a BMC, jerking of the skin around the dorsal hind limb region, was scored for 6-consecutive 5-min intervals during the 30-min post injection period. The cumulative number of WDS and BMC were used in the data analysis.

CHAPTER III

3. Results

3.1. Experiment 1: Does progesterone decrease extracellular 5-HT concentration in the MBH of EB-primed OVX rats?

Extracellular 5-HT (in pg per 10 µl), sampled from the MBH, was compared from OVX rats treated with sesame seed oil, 2.5 µg EB, 25 µg EB, 2.5 µg EB plus 500 µg progesterone, or 25 µg EB plus 500 µg progesterone. Data are presented in Figure 3. Extracellular 5-HT from rats receiving either dose of EB and progesterone were at the limits of detectability and were not considered in statistical analysis. Rats receiving either EB treatment did not differ from rats receiving oil-treatment ($F_{2, 19} = 0.430, p > 0.05$). Neither the time of day nor the time of day by treatment interaction were significant (all $p > 0.05$).

3.2. Experiment 2: Does progesterone decrease the ability of 8-OH-DPAT to inhibit the lordosis reflex of OVX EB-primed rats?

Eighty-four OVX rats were injected with 10 µg EB followed 48 hr later with either 500 µg progesterone (EP) or 0.1 ml of oil (EO) and were tested for sexual receptivity with a 10 mount pretest. Rats with a L/M ratio of 0.7 or more were injected with saline or a dose of 8-OH-DPAT (0.05, 0.075, and 0.10 mg/kg, i.p.) and behavioral testing proceeded for 6 consecutive 5 min intervals. There were significant main effects of hormone ($F_{1, 76} = 70.03, p \leq 0.0001$; Figure 4), dose of 8-OH-DPAT ($F_{3, 76} = 16.49, p \leq 0.0001$), and significant interactions between dose and hormone ($F_{3, 76} = 2.87, p \leq 0.041$), time and hormone ($F_{6, 456} = 5.32, p \leq 0.0001$) and time and

FIGURE 3

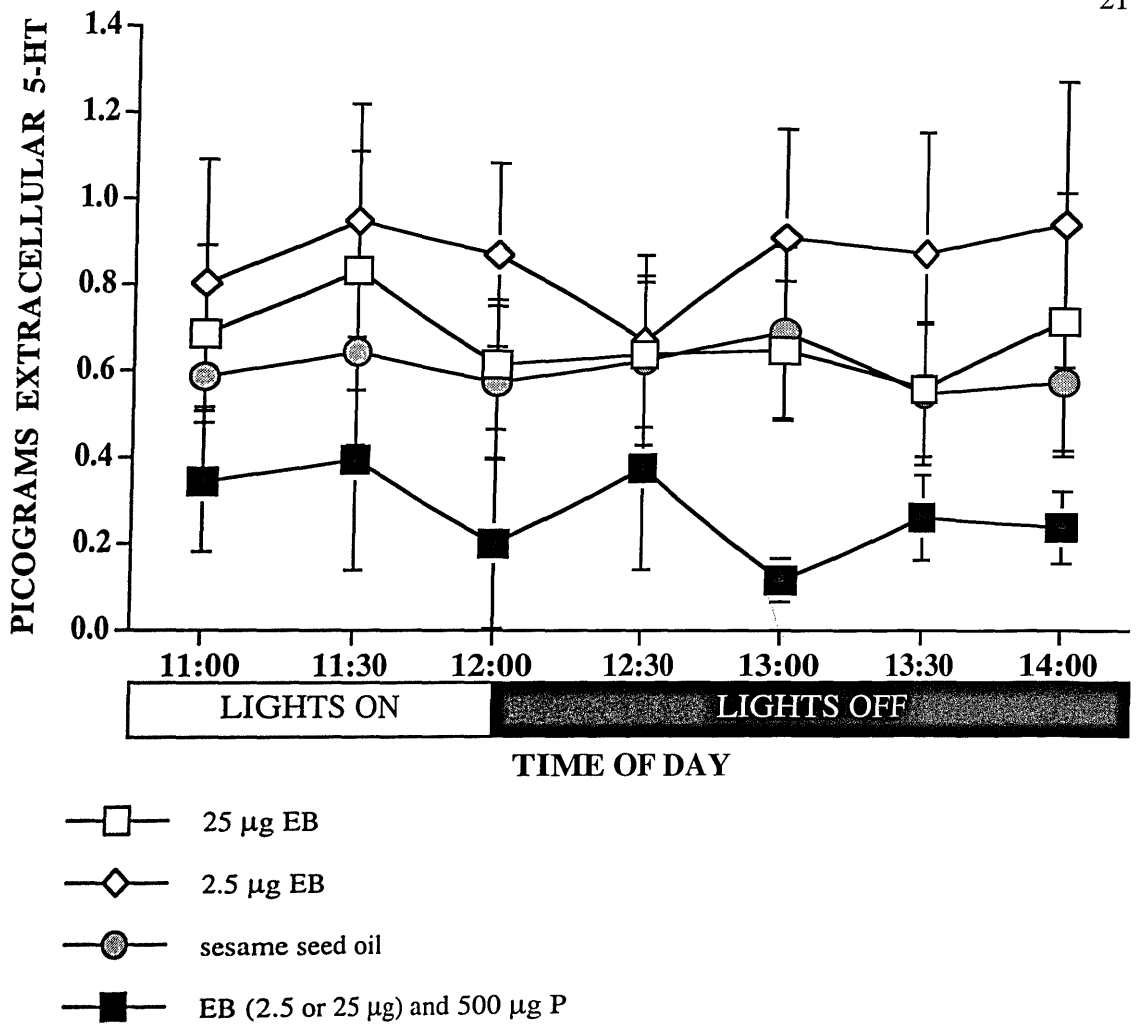


Figure 3: Hormonal regulation of extracellular 5-HT of the MBH. OVX rats were treated with 25 µg EB (n = 8), 2.5 µg EB (n = 9), sesame seed oil (n = 5) or 2.5 or 25 µg EB followed 48 hr later with 500 µg progesterone (n = 5). Microdialysis was initiated 2 days after treatment with EB or oil. Consecutive samples were collected via microdialysis from the MBH every 30 min from 11:00 am to 2:00 pm, with lights out at 12:00 noon. The means ± s.e. picograms of extracellular 5-HT per 10 µl sample are shown in the figure.

FIGURE 4

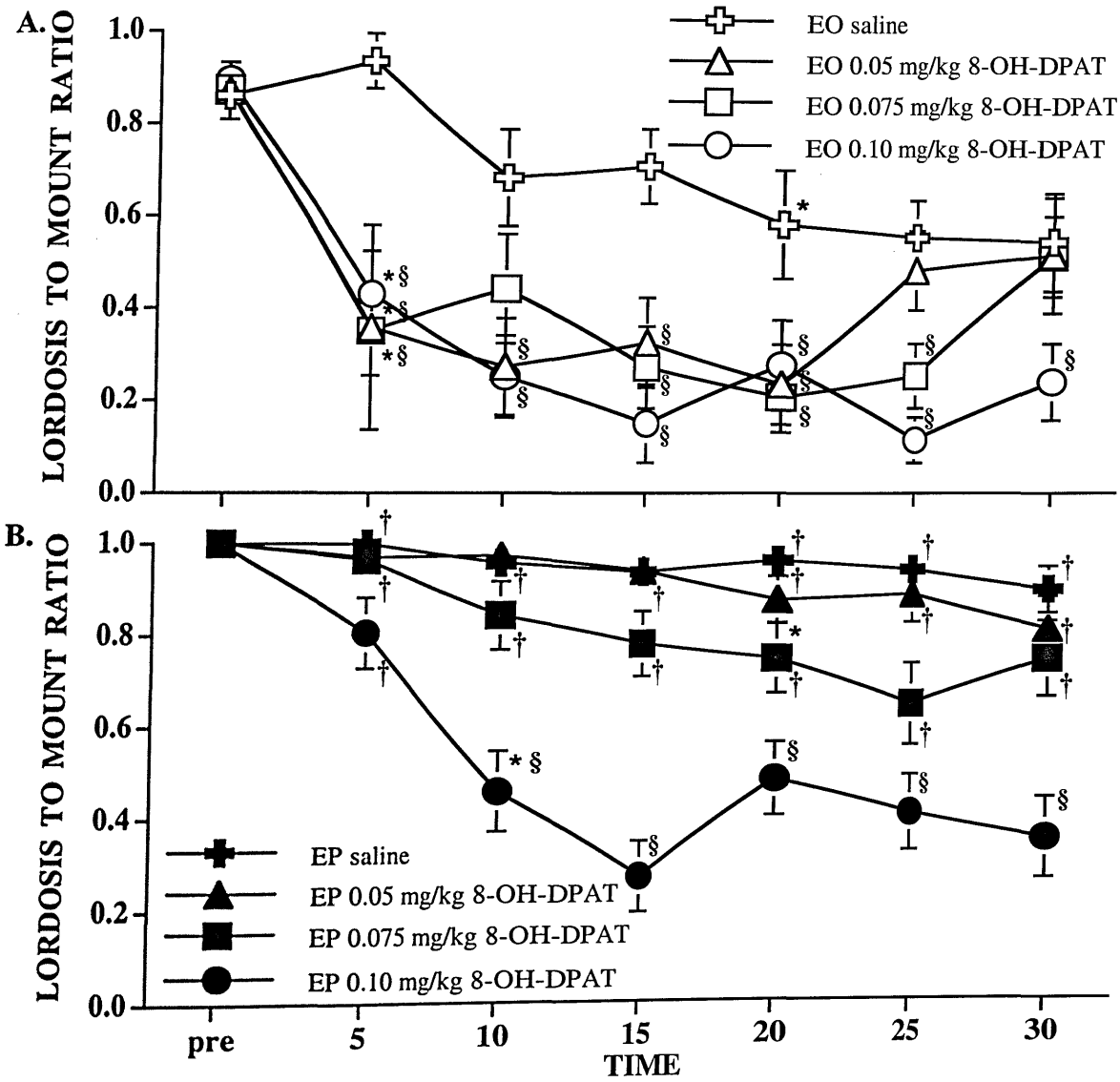


Figure 4: Modulation of 8-OH-DPAT-induced inhibition of the lordosis reflex by progesterone. OVX rats were treated with 10 μg EB followed 48 hr later by sesame seed oil [EO (see Figure 4A)] or by 500 μg progesterone [EP (see Figure 4B)]. Data are the mean ± s.e. L/M ratios for pretest and six consecutive 5 min intervals after injection with either saline (EO n = 5, EP n = 8), one of three doses of 8-OH-DPAT [0.05 mg/kg (EO n = 5, EP n= 11), 0.075 mg/kg (EO n = 13, EP n = 16), or 0.10 mg/kg (EO n = 12, EP n = 14)]. (*) = the first time interval that is significantly lower than pretest values (within treatment group) (†) = significant differences, between EO and EP rats receiving like injections, and (§) = significantly different from saline (within hormone condition).

dose ($F_{6,456} = 4.69, p \leq 0.0001$). In EO rats, all doses of 8-OH-DPAT significantly decreased the L/M ratio relative to the pretest within the first test interval after injection (Dunnett's, all $q_{456,9} \geq 2.38$, $p \leq 0.05$). Saline injections also produced a decline in the L/M ratios relative to pretest value, but not until the 20 min interval (Dunnett's $q_{456,5} \geq 2.16, p \leq 0.05$). Although saline's decline relative to pretest was significant in EO rats, all doses of 8-OH-DPAT produced a significantly larger decline in the L/M ratio compared to the decline produced by saline (Tukey's $q_{456,9} \geq 4.38$, all $p \leq 0.05$). In contrast to the 8-OH-DPAT effects in the EO rat, there was a dose-dependent effect of 8-OH-DPAT in EP rats. There was no significant decline in the L/M ratio after injections of saline or 0.05 mg/kg of 8-OH-DPAT relative to pretest (Dunnett's, all $q_{456,9} < 2.38$, $p > 0.05$). After the 0.075 mg/kg injection of 8-OH-DPAT, a significant decline in the L/M ratio relative to the pretest was present by the 20 min interval (Dunnett's $q_{456,16} \geq 2.42$, all $p \leq 0.05$). The 0.10 mg/kg dose of 8-OH-DPAT significantly decreased the L/M ratio by the 10 min interval (Dunnett's, all $q_{456,14} \geq 2.42$, $p \leq 0.05$).

Progesterone treatment significantly reduced the lordosis-inhibiting effects of 8-OH-DPAT ($F_{1,76} = 70.03$, $p \leq 0.0001$), with 8-OH-DPAT being more potent in EO rats. Doses of 0.05 and 0.075 mg/kg of 8-OH-DPAT produced significantly lower L/M ratios in EO rats compared to EP rats during all time intervals after injection, with the exception of 0.075 mg/kg dose at the 30 min interval (Tukey's, all $q_{456,9} \geq 4.38$, all $p \leq 0.05$). The 0.10 mg/kg dose of 8-OH-DPAT produced a significantly lower decline in EO rats compared to EP rats during the 5 min interval (Tukey's $q_{456,9} = 5.39$, $p < 0.05$). Unlike the EO rats, the L/M ratios of rats injected with either 0.05 or

0.075 mg/kg of 8-OH-DPAT were not significantly different from the L/M ratios of rats injected with saline (Tukey's, all $q_{456,9} < 4.38$, all $p > 0.05$). However, the L/M ratios of EP rats injected with 0.10 mg/kg of 8-OH-DPAT were significantly lower than the L/M ratio of EP rats injected with saline (Tukey's, all $q_{456,14} \geq 4.38$, all $p \leq 0.05$). After saline injection, EP rats had significantly larger L/M ratios than did EO rats from the 20 min interval on (Tukey's, all $q_{456,9} \geq 4.38$, all $p \leq 0.05$).

3.3. Experiment 3: The role of progesterone in the attenuation of 8-OH-DPAT-induced inhibition of the lordosis reflex by EB-priming.

The ability of a prior exposure of EB to decrease the lordosis-inhibiting effect of 8-OH-DPAT ± progesterone was assessed in experiment 3. Sixty-two OVX rats were injected with 10 μg EB or oil 7 days after surgery. Seven days after the initial injection all rats were injected with 10 μg EB followed 48 hr later by either an injection of 500 μg progesterone or oil. This hormone regimen produced 4 groups of rats: rats receiving a single dose of EB ± progesterone (OEP, OEO) and rats receiving two doses of EB ± progesterone (EEP, EEO). After a pretest for sexual receptivity, rats were injected with either 0.15 mg/kg or 0.5 mg/kg 8-OH-DPAT and testing continued for 30 consecutive min (Figure 5). The effect of progesterone alone was not significant ($F_{1,54} = 1.30, p \leq 0.259$), but the main effect of dose of 8-OH-DPAT was significant ($F_{1,54} = 21.56, p \leq 0.0001$). The 0.15 mg/kg dose of 8-OH-DPAT significantly inhibited the L/M ratios relative to their pretest at all time intervals in OEO, OEP and EEO rats (Dunnett's, all $q_{324,8} \geq 2.37$, all $p \leq 0.05$); EEP rats had only a transient decline in the L/M ratio during the 10 and 15 min intervals (Dunnett's, all $q_{324,8} \geq 2.37$, all $p \leq 0.05$). All rats that received the 0.5 mg/kg dose of 8-OH-DPAT had a significant

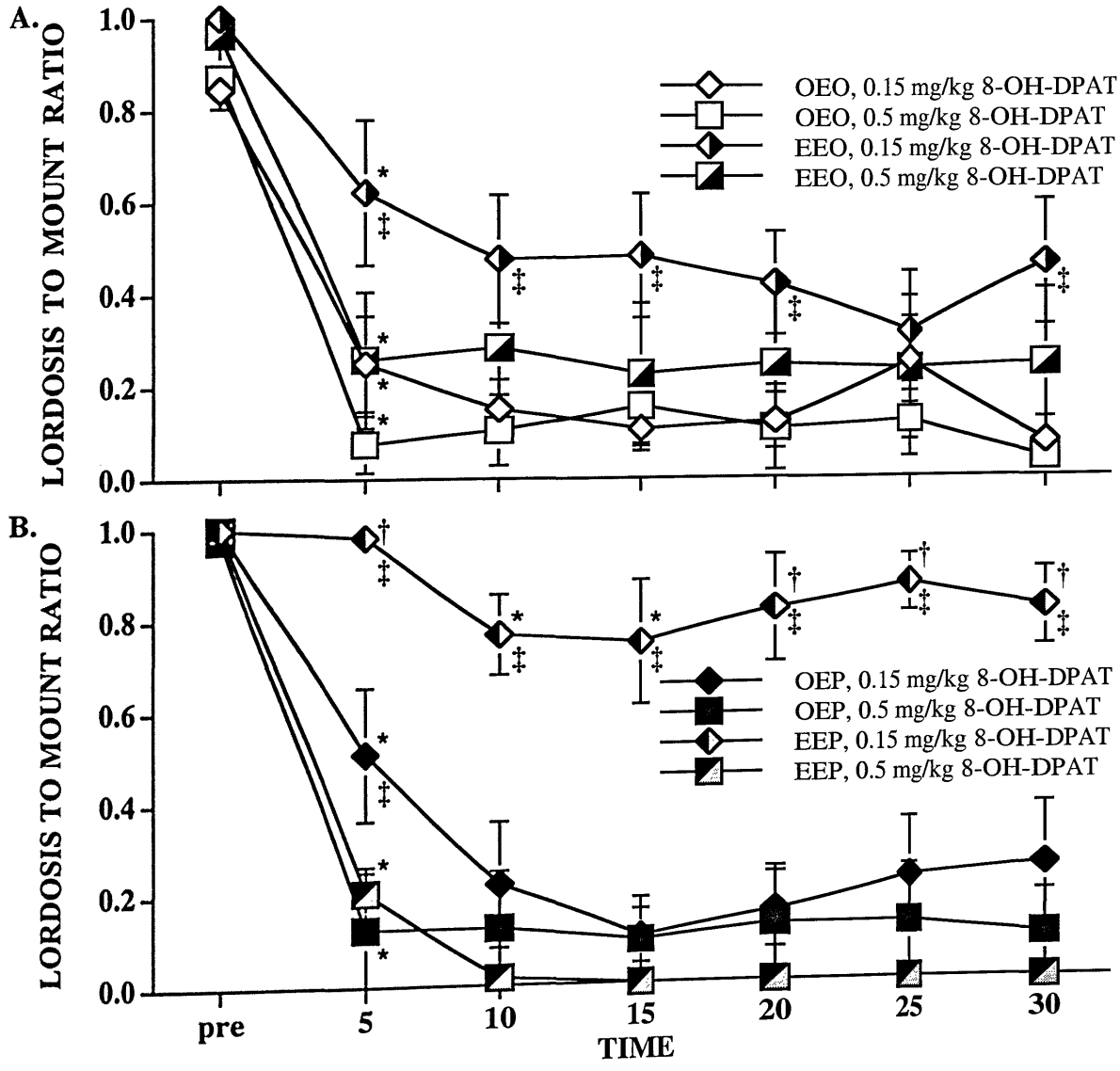


Figure 5: Effects of pretreatment with EB on modulation of 8-OH-DPAT-induced inhibition of the lordosis reflex in the presence or absence of progesterone.
OVX were primed with a single treatment of EB (10 μg) ± progesterone (OEO, OEP) or two EB treatments (10 μg) separated by seven days ± progesterone (EEO, EEP). Figure 5A represents the data for OEO and EEO rats, and Figure 5B represents data for OEP and EEP rats. Presented are the mean ± s.e. L/M ratios for pretest and six consecutive 5 min intervals after injection with either 0.15 mg/kg of 8-OH-DPAT (n = 8 for all groups) or 0.50 mg/kg injection i.p. of 8-OH-DPAT (n = 8 for OEO and OEP, n = 7 for EEO and EEP). (*) = the first time interval that is significantly lower than pretest values (within treatment group), (†) = significant differences in rats differing only in progesterone-priming and (‡) = significantly different L/M ratios in rats differing only in EB-pretreatment.

decline in the L/M ratio relative to pretest values at all time points after injection (Dunnett's, all $q_{324,8} \geq 2.37$, all $p \leq 0.05$).

Prior EB treatment significantly reduced the response to 8-OH-DPAT ($F_{1,54} = 15.22, p \leq 0.0003$). Except for the 25 min interval, EEO rats had significantly larger L/M ratios than OEO rats following 0.15 mg/kg 8-OH-DPAT (Tukey's, all $q_{324,8} \geq 4.36$, all $p \leq 0.05$); EEP rats had significantly larger L/M ratios than did OEP rats at all time intervals after 0.15 mg/kg 8-OH-DPAT (Tukey's, $q_{324,8} \geq 4.36$, all $p \leq 0.05$). Rats given prior EB treatment plus progesterone had significantly larger L/M ratios after the 0.15 mg/kg dose of 8-OH-DPAT ($F_{1,54} = 6.35, p = 0.014$) than rats given EB and oil after prior EB treatment during all time points except the 10 and 15 min intervals (Tukey's, all $q_{324,8} \geq 4.36$, all $p \leq 0.05$). The dose by number of EB treatments interaction ($F_{1,54} = 10.27, p \leq 0.0023$), time by dose by number of EB treatments interaction ($F_{6,324} = 3.10, p \leq 0.0057$) and time by dose by number of EB treatments by progesterone treatment interaction ($F_{6,324} = 2.41, p \leq 0.026$) were all significant.

Dose-dependent effects of 8-OH-DPAT's inhibition on the lordosis reflex are presented in Figure 4. Mean ± s.e. L/M ratios during the 10 to 25 min intervals of rats primed with a single treatment of EB ± progesterone were calculated from data in Figures 2 and 3 and are presented as in Figure 6 A. For each animal, the total number of lordotic responses during the 10 to 25 min interval after injection were divided by the total number of mounts. As represented in Figure 6 B, a higher dose of 8-OH-DPAT was required to decrease the L/M ratio in EP rats compared to EO rats, but the larger

FIGURE 6

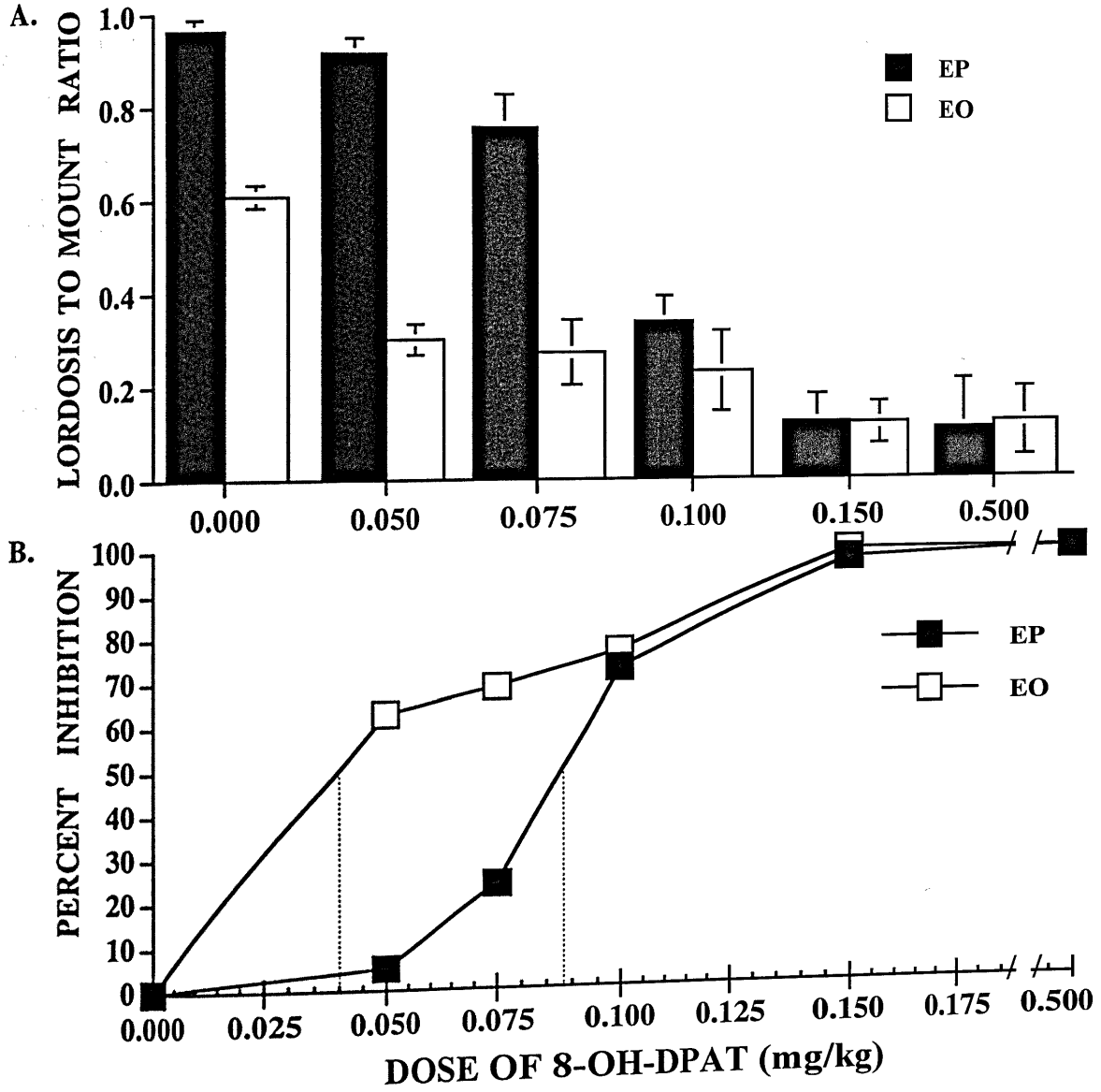


Figure 6: Progesterone reduces the potency of 8-OH-DPAT. Presented is a compilation of data from Figures 2 and 3. OVX rats receiving a single injection of 10 μg EB and oil (EO, open figures and bars) or a single injection of 10 μg EB and 500 μg progesterone (EP, closed figures and bars) 48 hr later, followed by an injection of saline or one of 5 doses of 8-OH-DPAT, were included in this figure. (A) The presented are the mean ± s.e. L/M ratios of EO and EP rats 10 to 25 min after injection with saline or 8-OH-DPAT. (B) Presented is the mean percent of maximum inhibition during the 10 to 25 min intervals after injection by dose of 8-OH-DPAT. Dotted lines represent an approximate ED50 for 8-OH-DPAT.

doses of 8-OH-DPAT, 0.15 and 0.5 mg/kg, produced a similar decline in the L/M ratio of both hormone treatment groups.

A dose-dependency curve of percent inhibition by dose of 8-OH-DPAT was constructed from the above data using rats given a single EB priming ± progesterone. Percent inhibition was defined for each hormone group independently by subtracting the average L/M ratio 10 - 25 min after injections of each dose of 8-OH-DPAT from the average L/M ratio 10 - 25 min after saline injection, and dividing that value by the maximum inhibition of each hormone group and multiplying by 100. Maximum inhibition was defined as the difference between the average L/M ratio of rats 10 to 25 min after injections of 0.5 mg/kg 8-OH-DPAT and the average L/M ratio of rats 10 - 25 min after injections of 0.0 mg/kg 8-OH-DPAT (saline). Dotted lines represent the approximate ED_{50} for each hormone group; the approximate ED_{50} of EP rats (0.090 mg/kg) is over two fold greater than that of the EO rats (0.040 mg/kg).

3.4. Experiment 4 : Does progesterone decrease the ability of VMN infusions of 8-OH-DPAT to inhibit the lordosis reflex in EB-primed OVX rats?

Forty-four OVX rats, implanted with VMN guide cannulae, were injected with 10 μg EB followed 48 hr later with 500 μg progesterone (EP) or 0.1 ml of oil (EO) and tested for sexual receptivity with a 10 mount pretest. Rats with a L/M ratio of 0.7 or more were infused with saline, 50 ng of 8-OH-DPAT or 100 ng of 8-OH-DPAT and behavioral testing proceeded for 6 consecutive 5 min intervals (Figure 7). Over the dose range examined, progesterone did not attenuate the inhibition of VMN infusions with 8-OH-DPAT ($F_{1, 38} = 0.54, p \leq .466$). Compared to saline infusions, both doses of 8-OH-DPAT examined decreased the L/M ratio regardless of hormone treatment

FIGURE 7

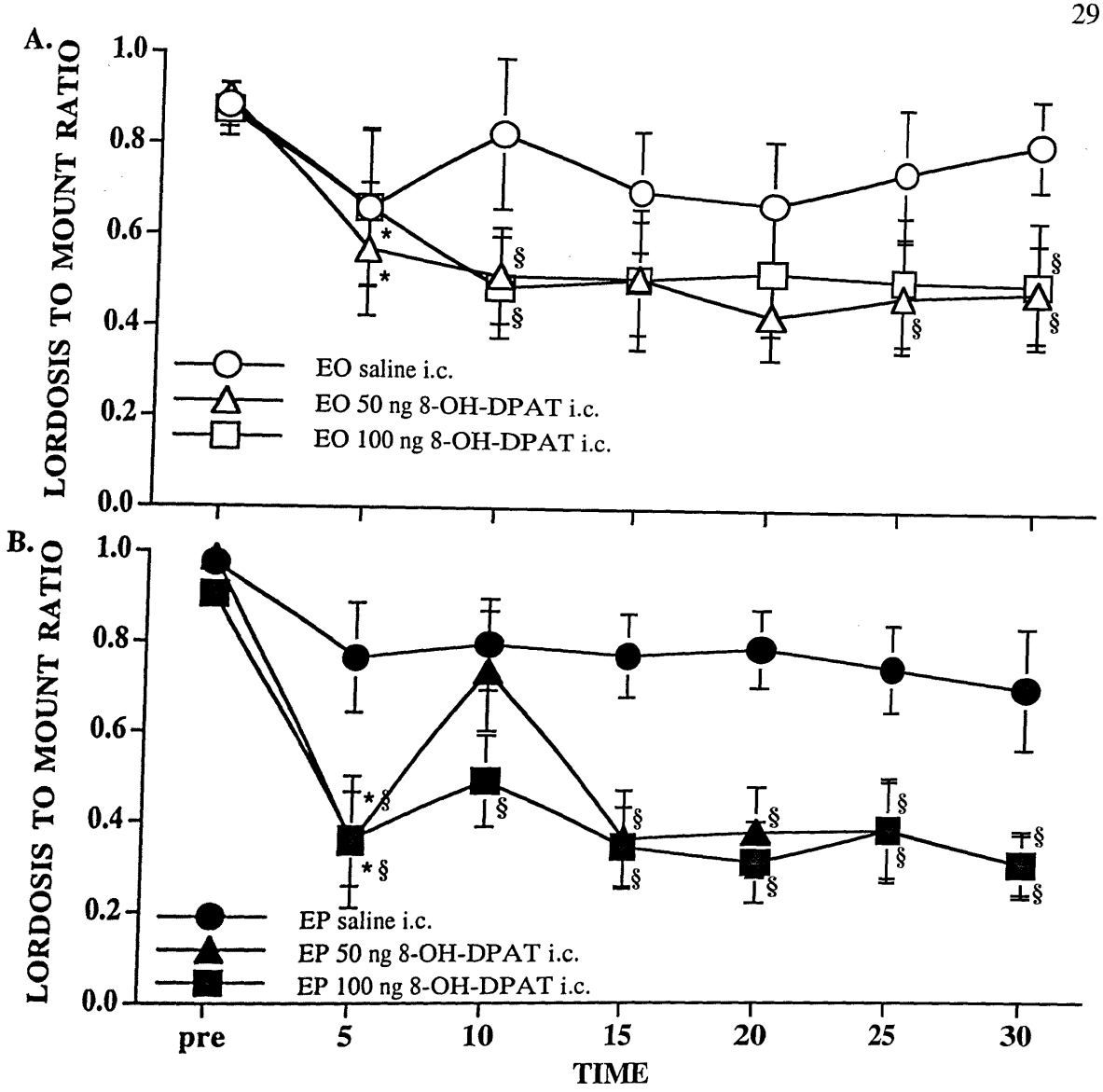


Figure 7: Effects of progesterone on modulation of lordosis inhibition by VMN infusions with 8-OH-DPAT.

OVX rats were treated with 10 μg EB followed 48 hr later by sesame seed oil [EO (see Figure 7A)] or by 500 μg progesterone [EP (see Figure 7B)]. Presented are the mean ± s.e. L/M ratios for pretest and six consecutive 5 min intervals after VMN infusion with either saline (EO n = 6, EP n = 7) or one of two doses of 8-OH-DPAT [50 ng (EO n = 8, EP n= 7) or 100 ng (EO n = 5, EP n = 11)]. (*) = the first time interval that was significantly lower than pretest values (within treatment group) and (§) = significantly different from saline (within hormone group).

($F_{2, 38} = 5.47, p = 0.008$, Tukey's $q_{228, 3} \geq 3.35$, all $p \leq 0.05$). This difference between L/M ratios after the saline infusion and L/M ratios after 8-OH-DPAT infusions appeared at an earlier time interval in EP rats compared to EO rats. There was no difference between dose of 8-OH-DPAT (Tukey's all $q_{38, 2} = 0.641$, all $p > 0.05$); both doses of 8-OH-DPAT led to a decline in the L/M ratios compared to pretest values ($F_{6, 228} = 19.91, p \leq 0.0001$). In EP rats, this decline occurred within the first 5 min after infusion of either dose of 8-OH-DPAT and lasted the duration of the experiment, with the exception of the 10 min interval of rats infused with 50 ng of 8-OH-DPAT (Dunnett's all $q_{228, 5} \geq 2.16$, all $p \leq 0.05$). In EO rats, both doses of 8-OH-DPAT significantly decreased L/M ratios relative to pretest values at all intervals after infusion (Dunnett's all $q_{228, 5} \geq 2.16$, all $p \leq 0.05$). Saline infusion did not produce a significant decline in L/M ratios compared to pretest values in either group of rats (Dunnett's, all $q_{228, 5} < 2.16$, all $p > 0.05$). No interactions were significant (all $p > 0.05$).

3.5. Experiment 5: Does progesterone increase 5-HT$_{2A/2C}$ receptor-mediated behaviors in EB-primed OVX rats?

The final experiment was designed to determine if progesterone would increase 5-HT$_{2A/2C}$ receptor-mediated behaviors in OVX, EB-primed rats. Twenty-four OVX EB-primed rats ± progesterone (EP, EO) were injected with either 1.5 mg/kg or 2.5 mg/kg of the 5-HT$_{2A/2C}$ receptor agonist, DOI. The number of WDS and BMC were recorded with a video camera for 6 consecutive 5 min intervals after injection. The cumulative mean number of observed behaviors (WDS and BMC) are plotted in Figure 8 A and B, respectively. Neither dose of DOI nor hormone condition had a significant effect on number of WDS (respectively, $F_{1, 20} = 1.88, p \leq 0.184$ and

FIGURE 8

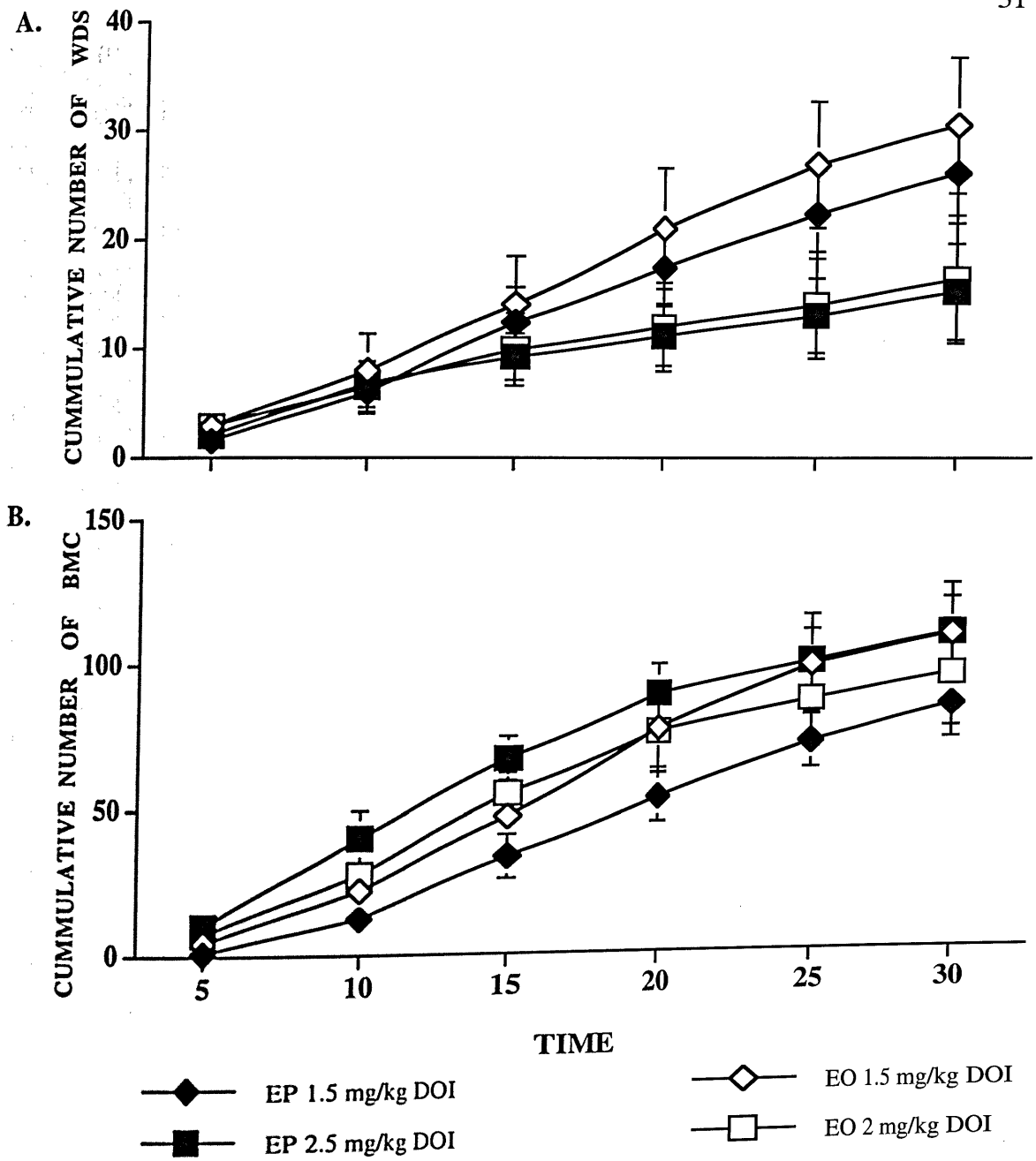


Figure 8: Modulation of 5-$HT_{2A/2C}$ receptor-mediated behaviors by progesterone. OVX rats treated with 10 μg EB and sesame seed oil (EO) or 10 μg EB and 500 μg progesterone (EP) were injected with 1.5 mg/kg or 2.5 mg/kg DOI (n = 6 for all groups), and the number of wet dog shakes (WDS) and back muscle contractions (BMC) were recorded for 6 consecutive 5 min intervals after injection. Presented in Figure 8A are the mean ± s.e. cumulative occurrences of WDS. Presented in Figure 8B are the mean ± s.e. cumulative occurrences of BMC

$F_{1, 20} = 0.27, p \leq 0.604$) or BMC (respectively, $F_{1, 20} = 2.90$, $p \leq 0.104$ and $F_{1, 20} = 0.09$, $p \leq 0.758$). For WDS, the time by dose interaction was significant ($F_{5, 100} = 10.09$, $p \leq 0.0001$), with rats receiving the 1.5 mg/kg dose of DOI displaying a greater number of WDS across time than those receiving the 2.5 mg/kg dose. The dose by hormone interaction, time by hormone interaction, and time by dose by hormone interaction were not significant for either number of WDS or BMC (all $p > 0.05$).

CHAPTER IV

4. Discussion

Progesterone's modulation of 5-HT and 5-HT receptor-mediated behavior was examined in the current project. In the first experiment, the effects of EB and progesterone on extracellular 5-HT of the MBH were examined using microdialysis and HPLC of freely moving OVX rats. Progesterone following EB treatment decreased extracellular 5-HT to the limits of detectability, while EB treatment of an OVX rat did not alter extracellular 5-HT levels compared to vehicle treatment. The decrease in extracellular 5-HT concentration after progesterone treatment is consistent with previous data utilizing microdialysis and tissue levels [22,15]. The current data, however, are the first to be reported in which the examination of the effects of EB and progesterone on extracellular 5-HT levels were performed in a freely moving rat. By using freely moving rats, the confounding variable of the anesthesia was eliminated. These data are also consistent with microdialysis data collected from intact rats. In these later studies, extracellular 5-HT of the MBH was found to be lowest in the intact rat during the phase of the estrous cycle that corresponds to a preovulatory progesterone surge [26,36].

There are several possible mechanisms by which hormones may alter extracellular 5-HT of the MBH. First, the reduction in extracellular 5-HT in the MBH after progesterone may be related to a decrease in the firing of 5-HT neurons which would subsequently lead to a decrease in release of 5-HT. Progesterone is readily metabolized in the rat brain into allopregnanolone, which potentiates membrane bound

$GABA_A$ receptors [52]. Drugs that activate $GABA_A$ receptors have been reported to decrease 5-HT neuronal firing [58]. Thus, the decrease in extracellular 5-HT in the MBH after progesterone priming may be a result of decreased 5-HT neuronal firing. A second mechanism by which progesterone may decrease extracellular 5-HT in the MBH is by enhancing activation of the 5-HT terminal autoreceptor, the $5\text{-}HT_{1B}$ receptor. Activation of the $5\text{-}HT_{1B}$ receptor on 5-HT neurons decreases the amount of 5-HT released per action potential [18], and Frankfurt et al. [17] reported an increase in $5\text{-}HT_{1B}$ receptors in the VMN of OVX rats treated with EB and progesterone compared to EB treatment alone. Hence, progesterone's reduction in extracellular 5-HT may be a consequence of increased terminal autoreceptor activation, decreased 5-HT firing via $GABA_A$ receptor enhancement, or a combination of the two.

The effects of EB on extracellular 5-HT were, however, unexpected. While no difference between extracellular levels of OVX EB-primed rats and OVX oil-primed rats was found, it was expected that EB-priming would lead to an increase in extracellular 5-HT. Lakoski [32] reported a decrease in $5\text{-}HT_{1A}$ receptor suppression of 5-HT neurons after EB-priming. Since activation of $5\text{-}HT_{1A}$ receptors on 5-HT neurons leads to a decrease in neuronal firing, it is not unreasonable to suspect that decreasing activation of these receptors would lead to an increase in 5-HT neuronal firing and consequently an increase in 5-HT release. Thus, it was predicted that EB-priming would lead to an increase in extracellular 5-HT. The absence of the expected increase in dialysate 5-HT after EB-priming may be explained by a report from McQueen et al. [39] who found that EB-treatment of an OVX rat increased serotonin transporter (SERT) binding sites in the MBH. SERT removes 5-HT from the synapse, decreasing

extracellular levels of 5-HT. If EB priming were to increase firing of 5-HT neurons, the effect on extracellular 5-HT in the MBH might be masked by increased uptake of 5-HT from the synapse. Further experimentation is required to better understand the absence of an effect of EB on extracellular levels of 5-HT.

The next set of experiments was designed to examine progesterone's ability to alter the lordosis-inhibiting effects of the 5-HT_{1A} receptor agonist, 8-OH-DPAT. Although there have been a number of reports on the effects of EB on 8-OH-DPAT-induced inhibition of the lordosis reflex, the effects of progesterone, until now, have not been systematically examined. The second experiment was designed to determine if progesterone priming would decrease the effectiveness of 8-OH-DPAT to inhibit lordosis. Since 5-HT inhibits lordosis primarily via activation of the 5-HT_{1A} receptor in the MBH, and progesterone decreases extracellular 5-HT in this area, it seemed likely that a greater dose of 8-OH-DPAT would be required to inhibit the behavior in rats primed with EB and progesterone (EP) compared to rats primed with EB alone (EO). 8-OH-DPAT was less effective at inhibiting the lordosis reflex in the EP rats when compared to the EO rats. However, EO rats that received a saline injection also had a reduction in the lordosis to mount ratio that was not present in EP rats receiving the same treatment. Thus, the increased effectiveness of 8-OH-DPAT reported in the EO rats may be confounded by the effects of the injection per se. Nevertheless, it is clear that EO and EP rats differ in their sensitivity to disruption of the lordosis reflex. Furthermore, relative to saline controls, there was a significant decrease in the L/M ratios produced by 0.05 and 0.075 mg/kg injections of 8-OH-DPAT in EO rats, but not in EP rats. Moreover, the potency of 8-OH-DPAT was found to be less in EP rats

relative to EO rats (see Figure 4), and potency estimates were computed relative to the appropriate saline control. Thus, the decrease in potency of 8-OH-DPAT in EP rats appears to be an effect of progesterone and not a reduced response to injection.

The attenuation of systemic injection of 8-OH-DPAT by progesterone closely resembles estrogen-induced attenuation of the effects of 8-OH-DPAT reported by Jackson et al. [28]. In this report, prepriming with EB increased the dose of systemic 8-OH-DPAT required to inhibit the lordosis reflex of OVX rats primed with EB and progesterone. Since, it is well known that EB can induce progesterone receptors in areas of the CNS involved in reproductive behavior [49], it was possible that the attenuation of 8-OH-DPAT by EB prepriming was related to progesterone's effect on 8-OH-DPAT's action. In the Jackson et al. [28] study, it was reported that attenuation of 8-OH-DPAT could occur without progesterone when rats were preprimed and primed with high doses of EB; however, the role of the later progesterone in the EB-prepriming effect was not directly examined. Consistent with previous research, in the current study, prepriming with EB decreased the effectiveness of 8-OH-DPAT to inhibit lordosis [28,57,60] independent of progesterone. However, the attenuation was even greater when EB-preprimed rats also received progesterone. Thus, progesterone, in addition to EB prepriming, counteracts 8-OH-DPAT-induced inhibition of the lordosis reflex.

The fourth experiment was designed to determine if progesterone would attenuate lordosis inhibition following VMN infusions with 8-OH-DPAT. 8-OH-DPAT is a potent inhibitor of the lordosis reflex when infused into the VMN, and inhibition of the lordosis reflex by 5-HT agonists is believed to occur at postsynaptic sites [2,34].

Considering that progesterone decreased the potency of systemic injections of 8-OH-DPAT, it was predicted that progesterone priming would also decrease the effectiveness of VMN infusion of 8-OH-DPAT. However, progesterone did not reduce the effects of VMN infusions with 8-OH-DPAT over the dose range of 8-OH-DPAT examined. The apparent lack of effect of progesterone priming on VMN infusions with 8-OH-DPAT may be a consequence of the dose range used. The effect of progesterone on systemic injections with 8-OH-DPAT was dose-dependent, so a wider range of concentration of 8-OH-DPAT infusions into the VMN may have unveiled a progesterone-induced shift in the potency of 8-OH-DPAT. Thus, progesterone's effect on VMN infusions of 8-OH-DPAT remains unclear.

It is well accepted that 5-HT has a dual role in lordosis behavior and that activation of 5-HT_{1A} receptors inhibits the behavior while activation of 5-HT_2 receptors facilitates the behavior [42]. Furthermore, effects of 5-HT_{1A} receptor activation can be reduced by 5-HT_2 receptor activation [62]. It has been hypothesized that progesterone enhances the functioning of 5-HT_2 receptors [64]. Thus, enhancement of the 5-HT_2 receptor functioning by progesterone may explain the decreased effectiveness of 8-OH-DPAT in inhibiting the lordosis reflex.

The effects of progesterone on 5-$HT_{2A/2C}$ receptor-mediated behaviors were examined in the last experiment. Differential effects of the 5-$HT_{2A/2C}$ receptor agonist, DOI, were not observed between hormone treatment groups. The addition of progesterone to an EB-primed rat did not alter the 5-HT_{2A} receptor-mediated behavior, WDS, or the 5-HT_{2C} receptor-mediated behavior, BMC, at either dose of DOI examined. An effect of dose of DOI, independent of hormone treatment, was observed

for the induction of WDS; WDS were increased more with the lower dose of DOI, 1.5 mg/kg. This is consistent with other reports of DOI-mediated behaviors, where bell-shaped dose response curves have been obtained [51].

The mechanism by which progesterone decreases the potency of systemic treatments with 8-OH-DPAT remains unclear. The hypothesis that progesterone may decrease 8-OH-DPAT's effect by enhancing 5-HT_2 receptors was not supported by this research. However, the 5-$HT_{2A/2C}$ receptor-mediated behaviors that were examined in this research may have a mechanism of action unrelated to the lordosis reflex, and, therefore, this hypothesis cannot be ruled out either. Based on the current data, however, it is clear that progesterone does not have a generalized effect of enhancing all 5-HT_2 receptor-mediated behaviors. Furthermore, the decrease in 8-OH-DPAT's potency may not be due to a reduced functioning of 5-HT_{1A} receptors in the VMN. Both doses of 8-OH-DPAT infused into the VMN were equally effective in decreasing the lordosis to mount ratio in EP and EO rats. Consequently, the neural site(s) responsible for progesterone's decrease in effects of 8-OH-DPAT on the lordosis to mount ratio cannot be determined from the current studies. In fact, the possibility that the progesterone-induced decrease in potency of systemic 8-OH-DPAT is due to a peripheral effect of progesterone cannot be ruled out. For example, progesterone may decrease the effectiveness of 8-OH-DPAT by increasing its metabolism. If progesterone's effect were due to enhanced metabolism of 8-OH-DPAT, then progesterone might be expected to be less effective at attenuating 8-OH-DPAT when the drug is given as a subcutaneous (s.c.) injection rather than an i.p. injection. However,

in unpublished experiments, we found that progesterone attenuated the effects of both i.p. and s.c. injections of 8-OH-DPAT.

Although alternative mechanisms cannot be conclusively rejected, the present findings are most consistent with the hypothesis that the decrease in potency of systemic 8-OH-DPAT results from progesterone's decrease in extracellular 5-HT. Systemic 8-OH-DPAT activates both pre- and postsynaptic 5-HT_{1A} receptors. Activation of presynaptic 5-HT_{1A} receptors leads to a decrease in 5-HT neuronal firing which leads to a decrease in 5-HT release and a subsequent decrease in extracellular 5-HT. The inhibition of the lordosis reflex induced by systemic injection of 8-OH-DPAT should be considered as a combined effect of 5-HT_{1A} receptor activation by 8-OH-DPAT and endogenous extracellular 5-HT. When extracellular 5-HT levels are low, as in EP-primed animals, a larger dose of 8-OH-DPAT would be required to activate enough postsynaptic 5-HT_{1A} receptors to inhibit lordosis.

It is well accepted that hormones influence the serotonergic system, and the current study supports this. The role of progesterone in 5-HT and 5-HT receptor-mediated behaviors was specifically examined in the current study. It was confirmed that progesterone priming of an EB-primed, OVX rat leads to a decrease in extracellular 5-HT in freely moving OVX rats. Furthermore, this decrease in extracellular 5-HT may be responsible for the decreased ability of systemic injections with 8-OH-DPAT to inhibit the lordosis reflex after progesterone priming. Although protective effects of progesterone were not obvious when 8-OH-DPAT was applied to the VMN, progesterone attenuated both i.p. and s.c. injections of the drug. Therefore, it is unlikely that progesterone attenuated effects of 8-OH-DPAT by simply increasing its

degradation. We also suggested that the progesterone-induced decreased effect of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, may be related to increased functioning of 5-$HT_{2A/2C}$ receptors and that this increase in functioning opposes the lordosis-inhibiting effects of the 5-HT_{1A} receptors. In this experiment, however, no generalized increases in 5-$HT_{2A/2C}$ receptor-mediated behaviors were witnessed with progesterone priming. However, the 5-$HT_{2A/2C}$ receptor-mediated behaviors examined in this experiment may be part of a circuit that is independent from that of the lordosis reflex. Further support for this statement comes from a recent finding. Gorzalka and co-workers recently demonstrated that 5-HT_2 receptor antagonists did not attenuate stress-induced facilitation of lordosis behavior but did attenuate stress-induced facilitation of WDS [8]. Thus, a lack of progesterone effect on the WDS behavior may not be relevant to progesterone's effect on 5-HT_2 receptors that are involved in the control of lordosis behavior. Thus, experiments to look directly at progesterone's effects on 5-HT_2 receptor control of lordosis should be done before completely discounting 5-$HT_{2A/2C}$ receptors as contributors to progesterone's attenuation of 8-OH-DPAT. However, since the progesterone-induced decrease in effects of 8-OH-DPAT may be explained by progesterone-induced alteration of extracellular 5-HT, studies to further pursue this possible mechanism should receive the highest priority. In conclusion, progesterone does play a role in modulating 5-HT and 5-HT receptor-mediated behavior, but more studies to explore the possible mechanism of progesterone's action are required.

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