FACILITATION OF LORDOSIS IN THE FEMALE RAT BY MIDBRAIN CENTRAL GRAY INFUSIONS OF SUBSTANCE P

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Facilitation of Lordosis in the Female Rat by Midbrain Central Gray Infusions of Substance P

by

© Wayne Allan Doman

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science

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ABSTRACT

Infusions of LHRH (50ng), and Substance P (50ng, 500ng, and lug) into the midbrain central gray led to a prompt facilitation of lordosis which was evident as early as 5 minutes post infusion, and depending on the dose had a duration of at least 120 minutes. Despite an apparent trend, different doses of substance P did not produce a signifcant dose-dependent facilitation of lordosis. Doses of substance P (50ng), and LHRH (50ng), produced effects on lordosis scores, but did not differ between tests. Administration of an anti-serum raised in rabbits against substance P failed to produce a statistically significant reduction in lordosis scores, although here too, a trend was evident.

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The expression of sexual behavior in the female rat has been unequivocally demonstrated to be dependent on ovarian hormones (Beach, 1948; Pfaff, 1970; Young, 1961). Removal of the ovaries results in complete cessation of sexual receptivity, and subsequent treatment with estrogen, or estrogen plus progesterone will reinstate mating activity (Pfaff, 1970). Although moderate levels of female sexual behavior can be elicited with estrogen alone, estrogen appears to act synergistically with progesterone to produce maximal responsiveness (see Pfaff, 1980 for review).

At present, the nature of the neural substrate of female sexual behavior remains unclear. However, areas within the hypothalamus, as well as extrahypothalamic structures have been implicated. Before reviewing the importance of these areas, a brief description of a sexually receptive female seems warranted.

A sexually receptive female rat responds to the male's mounting by assuming a stationary posture, in addition to a deep arching of the back (see Pfaff, 1980 for review). This stereotypical behavior results in exposure of her genital region, which subsequently facilitates thrusting probes by

the male and, ultimately, multiple intromissions followed by ejaculation (Bermant and Davidson, 1974). This posture is termed lordosis, and its occurrence is commonly used as a behavioral index of sexual receptivity.

Although lordosis is the most commonly used measure, less conspicuous behaviors can be observed during the receptive phase, termed estrus. For example, Beach (1976) coined the term "proceptivity" to designate the various active behaviors displayed by the female toward the male during behavioral estrus. Intensely receptive females will often manifest "hopping and darting" when in close proximity to a male. These behaviors have been reported to solicit sexual activity from the male (McClintock and Adler, 1978). McClintock and Adler describe this "solicitation" as having three distinct main components: i) approach , here the female approaches the male to within approximately two of his body lengths; ii) orientation, where the female orientates herself in a variety of ways around the male; iii) runaway, characterized by hopping and darting movements. The above behaviors are quite distinct from the more commonly used measure of sexual receptivity, lordosis; nonetheless, they play an important part

in the sexual activity of the female. However, studies concerned mostly with lordosis will be discussed. Later in the text, the effects of electrical stimulation, lesion, and infusion studies on lordosis will be discussed in more detail. Before proceeding further, however, it is necessary to introduce the areas of the brain believed to play a significant role in the induction or modulation of female sexual behavior.

The Importance of the Ventromedial Nucleus of the Hypothalamus (VMH).

Implantation studies:

As mentioned previously, estrogen treatment followed by progesterone is essential to produce maximum sexual receptivity in ovariectomized female rats (Beach, 1948; Young, 1961). In addition, estrogen implants into the brain via chronic cannula if followed by subcutaneous injections of progesterone, can facilitate lordosis. The VMH appears to be the most sensitive site of administration although estrogen implants into other areas of the brain can facilitate lordosis (Barfield and Chen, 1977; Yanase and Gorski, 1976). Minute quantities of estrogen placed in the VMH can induce lordosis even when placements of identical amounts in other areas produce little or

no behavioral effect (Barfield and Chen, 1977; Dorner, Docke, and Moustafa, 1968). To summarize, although other areas can produce facilitation of lordosis after central administration of estrogen, the VMH appears to be the most sensitive site; estrogen concentrations which are ineffective in inducing receptivity in other areas, induce receptivity when placed in the VMH.

Electrophysiological and biochemical evidence:

It has been demonstrated that the electrical activity of the hypothalamus varies during the estrous cycle of the female rat (Cross and Dyer, 1971; Dyer, Pritchett, and Cross, 1972; see Pfaff, 1973 for review). For example, Bueno and Pfaff (1976) recorded the activity of single neurons in the medial basal hypothalamus and preoptic area of urethane-anesthetized ovariectomized rats. They found that in the estrogen treated rats there was a greater number of cells in the VMH with spontaneous activity. Thus, it appears that treatment with estradiol has significant electrophysiological effects on VMH neurons. Although this increase in activity was observed after estrogen treatment, this effect may not be observed during the normal hormonal cycle of the animal, in light of a recent report (Chan,

Dudley, and Moss 1983). In that study they found that in untreated females the spontaneous electrical activity of VMH neurons was unaffected during the estrous cycle of the female, although estrogen levels do fluctuate during this period.

Nevertheless, since estrogen seems to increase electrical activity of neurons in and around the VMH, and central implant studies show that the estrogenic induction of receptivity is mediated by VMH neurons, what would the effect be on lordosis if electrical activity in and around the VMH were eliminated? In other words, if the electrical activity of nerve cells in and around the VMH is important for the induction of receptivity, then drugs which interfere with the ability of nerve cells to generate action potentials should result in dramatic reductions of lordotic responsiveness when infused into the VMH. Indeed, Harlan, Shivers, Kow, and Pfaff, (1983a) report that intrahypothalamic infusion of TTX, a drug which blocks voltage-dependent sodium channels by specific binding to the macromolecule containing the sodium channel (Narahashi, 1974), results in a dosage dependent, reversible decline in lordotic responsiveness. The administration of TTX led to an abrupt decline (within 6 minutes) in the

electrophysiological activity in these hypothalamic neurons which was followed by a concomitant decrease in lordotic responsiveness. Consequently, they were able to demonstrate for the first time a relationship between a reduction in multi-unit activity of hypothalamic neurons, and a lordosis deficit. In an earlier study, the same authors (Harlan et al., 1982) were able to show that an intrahypothalamic infusion of colchicine (a drug which inhibits axonal transport) into the VMH caused a dramatic decrement in lordosis.

Based on these studies, Harlan et al. proposed a working model for estrogen's action on lordosis-relevant cells in the hypothalamus. Since estrogen is known to stimulate protein synthesis in the hypothalamus (McEwen et al., 1979), increased protein or peptide synthesis in the VMH, which is presumably estrogen induced, could theoretically lead to an increase in transported substances from the VMH to other parts of the brain, particularly the midbrain, where they could be released from nerve terminals to act on midbrain cells.

Lesion and knife cut studies:

Lesions in and around the VMH led to decreased receptivity in female rats (Carrer, Asch, and Aron, 1973; Dorner, Docke, and Gotz,

1975; Edwards and Mathews, 1977) hamsters (Malsbury, Kow, and Pfaff, 1977), sheep (Domanski, Przekop, and Skubiszewski, 1972), and cats (Hagomen and Brooks, 1958). In fact, Malsbury et al. (1977) demonstrated that in female hamsters, the degree of the lordosis deficit is positively correlated with the degree of VMH damage. In a study published by Malsbury and Daood (1978) it was concluded that in the female hamster, neural pathways leaving the VMH in an antero-lateral direction are critical for lordosis (see Malsbury, Miceli, and Scouten, 1984 for review). The critical importance of these pathways was later confirmed in the rat (Pfeifle, Shivers, and Edwards, 1980), where sagittal-plane knife cuts placed lateral to the VMH severely attenuated the occurrence of lordosis. Moreover, although most lesions of the VMH produce decrements in lordosis, a more profound effect is observed when the lesions are placed on the lateral side of the VMH (Pfaff and Sakuma, 1979). Taken together, these reports lend strong credibility to the idea that VMH efferents (which project mediolaterally out of the hypothalamus) are important for the neuronal control of female sexual behavior.

Electrical stimulation:

If estrogen implants in the VMH induce receptivity, and VMH lesions or knife cuts of VMH connections disrupt this receptivity, it follows that electrical stimulation of the VMH should facilitate lordosis in estrogen primed female rats. Indeed, Pfaff and Sakuma (1979b) reported that when electrodes were placed in the VMH, gradual increases in lordosis scores were observed following relatively long periods of stimulation. For example, a minimum of 15 minutes, but more often 60 minutes of stimulation was required before maximum receptivity was observed (see Pfaff, 1980 for review). A rather interesting point from that study (which will become relevant later in the text), was that for optimal responsiveness the electrodes had to be placed on the lateral side of the VMH.

Importance of the VMH: Conclusion.

Studies in the rat and hamster strongly suggest that neurons in and around the VMH facilitate lordosis. For example, partial removal of the VMH by lesions (Edwards and Mathews,1977; Pfaff and Sakuma, 1979a; Malsbury et al., 1977), or knife cuts of VMH connections (Malsbury and Daood, 1978), disrupt lordosis. In addition, estrogen implants (Barfield and Chen, 1977), or

electrical stimulation in the VMH (Pfaff and Sakuma, 1979b), facilitate lordosis. Moreover, drugs that either disrupt action potentials (Harlan et al., 1983) or axonal transport (Harlan, Shivers, Kow, and Pfaff, 1982) in the VMH, led to dramatic reductions in lordosis responsiveness. Taken together, these data strongly suggest the importance of the VMH in female sexual behavior. They also suggest that VMH efferents travelling in an antero- lateral direction, are critical for lordosis. Consequently, since a major projection of the VMH is to the midbrain central gray via a direct periventricular projection, and a more lateral one (Krieger, Conrad, and Pfaff, 1979), and partial disruption of the VMH input to the central gray leads to lordosis deficits in female hamsters (Malsbury and Daood, 1978), the midbrain central gray (MCG) appears to be an excellent candidate for a role in modulating female sexual behavior. Therefore, a brief introduction to experimental support for this role seems warranted.

The Importance of the MCG:

Since decerebrate female rats with complete transection between the diencephalon and mesencephalon which interrupts important hypothalamic descending pathways do not display

lordosis, efferents descending from the hypothalamus may be critical for lordotic responsiveness in female rats. For example, anterior to these transections, no source of axons other than the VMH has been shown to play a role in the facilitation of lordotic responsiveness. Moreover, out of the four major projections of the VMH, two project to the MCG. One is a medial-periventricular projection, and the other, an antero-lateral projection, and disruption of this antero-lateral projection leads to drastic declines in lordosis responsiveness. Furthermore, in light of a recent report that estrogen concentrating cells in the VMH project directly to the MCG (Morrell and Pfaff, 1982), nerve cells in the VMH, as well as in and around the MCG, appear to be logical candidates for the descending output in lordosis. To address this issue further, however, requires a more detailed understanding of the role of the MCG in the control of female sexual behavior.

Pfaff, Lewis, Diakow, and Keiner (1972), reported that electrical stimulation of the midbrain central gray in anesthetized female rats results in behavioral movements resembling lordosis. This led them to postulate that MCG

neurons play a role in the organization of sexual behavior. Later, support for this came from a variety of studies. For example, knife cuts which disrupt VMH efferents to the MCG produce dramatic decrements in lordotic responsiveness in female hamsters (Malsbury and Daood, 1978). Also, it has been demonstrated that electrical stimulation of the central gray facilitates lordosis in female rats (Sakuma and Pfaff, 1979a), while electrolytic lesions of this area produce immediate decrements in lordosis (Sakuma and Pfaff, 1979b). In the midbrain central gray lesion study done by Sakuma and Pfaff (1979b), they report that the most severe decrements in lordotic responsiveness were found when the lesions were placed in the dorsal segment of the midbrain central gray (a point that will be discussed in more detail later). The above studies are in contrast to a VMH study where very precise lesions within the VMH produced a gradual decrement in lordosis (Pfaff and Sakuma, 1979). In that study lesions were made through chronically implanted electrodes placed in the VMH. The lesions did not disrupt lordosis immediately, but led to a gradual decline in the lordosis reflex. Based on the above, it seems likely that the MCG, perhaps under the

influence of the midbrain central gray, plays an important role in the modulation of female sexual behaviour. Moreover, since a study which disrupted axonal transport from the VMH to the MCG (as well as in other VMH efferents) produced lordosis deficits in female rats (Harlan et al., 1982), it seems likely that axonal transport of substances from the VMH to the MCG would be critical for the maintenance of lordotic responsiveness. Although at present the chemical nature of these substances is unknown, there are recent reports in the literature which suggest a role for peptides in the modulation of sexual behavior.

The Role of Peptides in the Modulation of Sexual Behavior.

Luteinizing hormone releasing hormone (LHRH) a decapeptide (pGlu- His- Trp- Ser- Tyr -Gly- Leu- Arg- Pro- Gly- NH2) is a hypothalamic regulator of luteinizing hormone (LH) in animals (Amoss, Blackwell, and Guillemin, 1972), and in humans (Schally, Kastin, Arimura, Coy, Redding, 1973). During the estrous cycle of the female rat, the ovulatory discharge of LH appears to be synchronized with behavioral receptivity (Beach, 1948; Schwartz, 1969; see Moss and Dudley, 1980, for review). In addition, subcutaneous injections

of LHRH have been shown to facilitate lordosis in estrogen primed female rats, an effect which is not dependent on the pituitary or the pituitary-adrenal axis (Pfaff, 1973). Using immunocytochemical methods several investigators have found LHRH containing axons which project to the MCG (Barry and Dubois, 1976; Silverman and Zimmerman, 1977). This raises the possibility that LHRH could facilitate lordosis at the level of the central gray. Indeed, Riskind and Moss (1978), demonstrated that infusion of 50ng of LHRH into the MCG of female rats enhanced lordosis. This facilitation was, however, dependent on estrogen in as much as females had to receive a minimum of 5ug of estrogen subcutaneously 96 hours before infusion of the peptide.

Recently, an elaborate series of studies has been published (Sakuma and Pfaff, 1983) which further substantiate the results reported by Riskind and Moss (1978) with LHRH. In these particular studies, not only did LHRH infusion into the midbrain central gray facilitate lordosis, but an LHRH antagonist led to profound deficits in lordosis. This led Sakuma and Pfaff to postulate that LHRH has excitatory effects on MCG neurons, and that the origin of the LHRH is the VMH.

Although otherwise an attractive hypothesis, immunocytochemical evidence concerning LHRH containing cells in the mediobasal hypothalamus remains somewhat controversial (Clayton and Hoffman, 1979; Kozlowski and Dees, 1984). In fact, in the working model proposed by Harlan et al., 1983 (previously discussed), it was concluded that of the neuroactive products which are transported to the MCG from the VMH and probably essential for lordosis, LHRH is an unlikely candidate. This was based on immunocytochemical studies which had revealed few LHRH-containing cells in the medial basal hypothalamus. Lastly, the behavior of the sexually receptive female that is observed after LHRH infusion does not resemble that of a fully receptive female. In other words, although the female displays lordosis, the proceptive component of the behavior is missing.

Interest in another peptide possibly involved in sexual behvior has emerged recently (Harlan et al., 1983). This study reports that infusion of prolactin into the MCG facilitates lordosis in female rats. In addition, prolactin-like immunoreactive cells were found in a band extending laterally from the arcuate nucleus

to just ventral to the VMH. Prolactin-like immunoreactive fibers were discovered throughout the MCG. This facilitation of lordosis observed after prolactin infusions adds another level of complexity. However, interpretation of these results is complicated by the report that prolactin has been shown to inhibit lordosis when infused into the lateral ventricles (Dudley et al., 1982). In addition, if fibers projecting from the VMH to the MCG are important for lordosis, one would expect to find immunoreactive cells in the VMH, as VMH cells seem to be most important for the estrogenic induction of receptivity (a point previously made). However, prolactin-immunoreactive cells were not found in the VMH itself. Furthermore, it was reported that the majority of immunoreactive fibers found in the MCG were located in the ventral portion, whereas other evidence suggests the importance of the dorsal area (Pfaff, 1980).

In conclusion, although the above studies suggest an important role for peptides in sexual behavior, interpretation of these results does not conclusively demonstrate a LHRH or prolactin VMH-central gray interaction. In order to make this claim certain criteria need to be met.

Firstly, the peptide must produce behavioral effects when administered. Secondly, cell bodies in the VMH should contain the peptide, since experimental evidence suggests that these cells are most sensitive to estrogen implants. Finally, receptors in the MCG, particularly in the dorsal aspect, which has been shown to be most sensitive to lesion or electrical stimulation, must be found for the peptide in question. Based on the above criteria, substance P appears to be a logical candidate.

Substance P

Substance P an undecapeptide (Arg -Pro -Lys- Pro -Gln -Gln -Phe-Phe- Gly- Leu-MetNH2) was first described by von Euler and Gaddum in 1931. They prepared a standard dry powder to do quantitative comparisons of active substances that they were investigating. The substance that they prepared was called substance P, which was an abbreviation for substance powder. This name has remained unchanged. In fact, in an opening address at a symposium on substance P held in London in 1981, A.S.V. Burgen remarked that " after all this time. I wonder if anyone will succeed in imposing a more sophisticated name on such a fascinating substance ".

Immunohistochemical analysis has shown that substance P is located in more than 30 cell groups in the central nervous system (Ljungdahl, Hokfelt, and Nisson, 1978). In fact, Ljungdahl et al., (1978) were able to demonstrate substance P-like reactivity in cell bodies in the VMH. Furthermore at the medial and posterior levels the VMH labeled cells were found in the ventral lateral portion (mentioned previously to be most sensitive to estrogen implants). In the same study, immunoreactive fibers containing substance P-like reactivity were found in the midbrain central gray. Not only is it encouraging that the MCG contains nerve terminals of substance P, but also that the highest densities of these were found in the dorsal portion.. This portion of the MCG is the most sensitive to electrical stimulation (a point that was discussed earlier in the text).

Further support for the candidacy of substance P comes from a recent study by Quirion, Shults, Moody, Pert, Chase, and O'Donohue, (1983). They report that a high level of substance P receptors was found in the MCG.

Based on the above, it seems possible that the undecapeptide substance P could be involved in the modulation of sexual receptivity in the female

rat. Therefore, it was the purpose of this thesis project to investigate the effect of midbrain infusions of SP on female sexual behavior.

METHOD

Subjects: Sixty adult female Sprague-Dawley rats, purchased from Charles River Breeding Farms, St. Constant, Quebec, Canada were used in this study. The animals weighed between 260-300 grams at the time of surgery. These animals were housed singly in solid bottom plastic cages (33x19x15 inches) in a controlled environment at 21 C, with a reversed light cycle (lights off 12:00 p.m., lights on 12:00 a.m.). Free access to food and water was maintained throughout the experiment.

Procedure: Animals were ovariectomized under Sodium Pentobarbital (Somnotol, 5.2mg/100gms) before stereotaxic surgery. After a one week recovery period, the animals were stereotaxically implanted with a pair of 22-gauge stainless steel guide cannulae with inner stylets (28 gauge) aimed at the dorsal midbrain central gray. The cannulae were acquired from Plastic Products, Roanoke, Va. The stereotaxic coordinates that were used were as follows: From bregma, AP:-6.3; DV:4.6; ML:0.5 (skull flat, from dura, right side); AP:-3.4; DV - 5.0; ML:-2.3 (skull flat, from

dura left side, 30° angle). The AP coordinate for the left side was calculated based on the 30° angle anterior to the animal's head. This angle resulted in a recalculation of the AP coordinate (see appendix A). On the day of cannulation all animals received 10ug of estradiol benzoate (Sigma) subcutaneously. This was done because it has been reported that after ovariectomy, there is a subsequent decline in estrogen receptors in the brain (Clark, Maclusky, Parsons, and Naftolin, 1981). One week later, each animal received a subcutaneous injection of 5ug estradiol benzoate in ethyl oleate, and was tested 96 hours later for sexual receptivity by pairing with a sexually vigorous male. Only animals which responded with moderate to low degrees of lordosis were used in the experiment. Animals chosen on this day were given a one week recovery period before peptide infusion. After the recovery period, animals that responded with moderate to low levels of receptivity on the initial screening day were given an additional 5ug of estradiol benzoate and tested with the peptides 96 hours later. As before, only animals that were moderate to low responders were used. Three days later, animals that did not satisfy the aforementioned criteria were given an

additional 5ug estradiol benzoate, and tested 96 hours later.

Intracerebral Infusion of Peptides:

All peptides were prepared on the first day of behavioral testing and stored in individual aliquots in a liquid nitrogen freezer (-40° Celsius). Saline which was used as the vehicle (acidified saline for SP), was sterilized using a Nalgene sterilization filter; cat. 120-0020. This was done prior to mixing of the peptides. On the day of testing, one alliquot containing each particular peptide was removed 15 minutes before the testing took place.

LHRH (Sigma, lot 43f-5860) was infused (the following procedure will be synonymous with "infusion" throughout the remaining text) bilaterally in unanesthetized animals in a volume of 0.5 ul (each side) over a period of 60 seconds through the internal cannula. The procedure was as follows. Immediately before peptide infusion, the animal was removed from its cage and the two dummy cannulae obturators removed. A 5 uL Hamilton syringe (cat. 7105) and an internal cannula joined by a 12 inch plastic tube were used. Sterilized distilled water was then drawn up the plastic tube until 1 uL of water could be reliably

drawn and expelled. Following this, a half microliter of air was drawn up into the internal cannula, whereupon the internal cannula was placed into the peptide solution and 4.5 ul of peptide was drawn. The movement of the air bubble enabled the investigator to determine if the peptide had actually been infused into the brain. If the air bubble failed to move, the entire procedure was repeated. The internal cannula was left in place for 15 seconds prior to withdrawal. The concentration of LHRH was 100ng in lul of saline (0.9%). This resulted in a dose of 50ng in .5ul for each side. This dose has been previously reported to be the most effective (Sakuma and Pfaff, 1983). Two sets of microsyringes were used to enable consecutive deliveries of the solution into each side of the brain. Infusions took approximately four minutes to complete. Each animal was tested within the first 5 minutes after the 2nd infusion.

Substance P (Sigma) was infused in essentially the same manner, however, three different concentrations were used: lug, 500ng, or 50 ng of peptide in 0.5ul of acidified saline. The pH's of the SP/acetic acid/saline solutions were 4.7. Since substance P is basic, the pH of the

acidified saline vehicle was 3.9. The acidified saline was made by preparing a 0.01 N solution of acetic acid and adding this solution to 0.9% saline (0.05 ml of acetic acid in 100 ml's of 0.9% saline). Although there are no reports in the literature concerning SP and sexual behaviour, a dose of lug of SP administered into the MCG was used in a study of substance P-induced analgesia in rats (Mohrland and Gebhart, 1979). Since there were no secondary behavioral effects reported, it was decided that lug would be the largest dose in this study. The rationale for using acidified saline was as follows: i/ it helps prevent adsorption to glass, and may make the peptide more stable; ii/ Hall and Stewart (1983) compared the behavioral effects of SP dissolved in saline or acidified saline injected intraperitoneally into mice. They found consistently stronger behavioral effects when SP was dissolved in acidified saline. Consequently, acidified saline was used as the vehicle in this experiment.

Testing Protocol:

Each animal received a subcutaneous injection of 5ug estradiol benzoate in ethyl oleate 96 hours prior to infusion of peptides or vehicle. Animals were tested 1 hour before infusion, within

5 minutes prior to infusion, within 5 minutes post infusion, and then at intervals of 5 minutes through the first 15 minutes, 15 minutes later, and then at 30 minute intervals until 5 hours post infusion. Testing began at 2:00 p.m. which was 2 hours into the dark period of the cycle.

Two behavioral indices of receptivity were used. The lordosis reflex score (LRS), (Pfaff, 1980), and the intensity of lordosis score (ILS) (Hardy and Debold, 1971). Using the LRS, the animal receives manual cutaneous stimuli to the flanks which are followed by pressure on the rump-tail base-perineum region (Pfaff et al., 1977). The LRS is established after manual stimulation has been delivered 5 times, whereupon the average score is taken. The reflex score consists of: 0 = no vertebral dorsiflexion, 1 = low, 2 = moderate, and 3 = strong dorsiflexion. The ILS uses a similar scale. However, here the female is placed with a male until the male has mounted, with pelvic thrusting, 10 times. This measure was not taken as frequently as the manually-elicited LRS. It was measured at -60 minute (one hour before infusion), approximately 5 minutes prior to infusion (0 test), at 30 minutes post infusion and then at one hour intervals until 4 1/2 hours post infusion.

After each infusion session, all animals were given a one week recovery. Animals which had been previously infused with substance P were subsequently given a different dose in a counterbalanced order. Animals which had received a control infusion, remained in the control group for the remainder of the experiment. This enabled the investigator to determine if control scores changed over the course of the experiment due purely to the familiarity of the test situation. Animals in the LHRH group were given only one test session with LHRH and then were given different doses of SP.

Eight animals which had successfully been infused with at least two different doses of substance P were placed in the antiserum group. In addition, 8 animals which had had at least two control infusions (vehicle) were placed in the anti-serum control group. This enabled the investigator to examine the physiological necessity of midbrain release of SP. All animals were placed on a high estrogen regime (10ug estradiol benzoate given subcutaneously for 5 consecutive days). On the afternoon of the fifth day, all animals were placed with a male and tested for receptivity. Animals displaying maximum ILS were subsequently

infused with undiluted rabbit antiserum to SP (Immunonuclear), or normal rabbit serum (control), bilaterally in the MCG and tested using the ILS. The volume of the infusion was 0.5 ul into each side. As a result of the high level of activity of highly receptive females, and the subsequent difficulty obtaining reliable LRS scores, only male stimulation was used for the anti-serum animals.

Histological Analysis

Upon completion of data acquisition, all animals were anesthetized with an overdose of sodium pentobarbital. All brains were subsequently removed, and 46 micron sections taken on the cryostat. Using a cresyl violet stain, each section was checked for cannula placement with a microprojector.

RESULTS

Histology:

Histological examination confirmed that 48 of 60 animals had both cannula tips within, immediately dorsal to, or immediately ventral to the dorsal central gray region. Analysis of the behavioral results is based on data from these 48 animals with dorsal midbrain implants. Five animals had only one cannula in the dorsal central

gray, while 3 animals had both cannulae located outside the central gray; data from these animals were not used in the statistical analysis. Four animals died before behavioral testing took place. Placements varied in their anterior-posterior location from 6.3 - 8.8 mm (Paxinos and Watson, 1982). Figure 1 is a composite diagram of the cannula placements.

LHRH EXPERIMENTS.

Infusion of LHRH into the dorsal midbrain produced a facilitation of lordosis in response to manual stimulation, and in response to mounts by male rats as compared to vehicle infusion (n=12 ; $F=13.07$, $p<0.01$, Table 1; $F=11.28$, $p<0.01$, Table 2, respectively). As shown in figure 2, in response to manual stimulation, this facilitation was observed as early as five minutes post infusion, reached its maximum at 30 minutes, and lasted for as long as 2 1/2 hours post infusion. Figure 3 illustrates the facilitation observed in response to male stimulation. In addition to showing a peptide effect, the analysis also revealed a time and a time by group interaction (tables 1 and 2). Post hoc analysis using a Newman-Keuls test revealed significant differences between groups at 5 minutes post infusion through

Figure 1 A Localization of cannula sites in the midbrain central gray. On left side of each section, sites which were effective in facilitating lordosis are indicated by asterisks. Filled circles on the right side indicate sites where infusions did not facilitate lordosis. Numbers in upper left corner of sections indicate rostral-caudal distance in mm from bregma.

This representation was done purely for graphical purposes; aq = cerebral aqueduct; CG = central gray; dr = dorsal raphe; DpMe = deep mesencephalic nucleus; ml = medial lemniscus; mcp = middle cerebellar peduncle; ppfg = pedunculopontine tegmental nucleus; rmc = red nucleus magnocellular; rpn = raphe pontis nucleus; vll = ventral nucleus lateral lemniscus.

B Illustrates the four divisions in the central gray used in descriptions throughout the text; MCG = midbrain central gray.

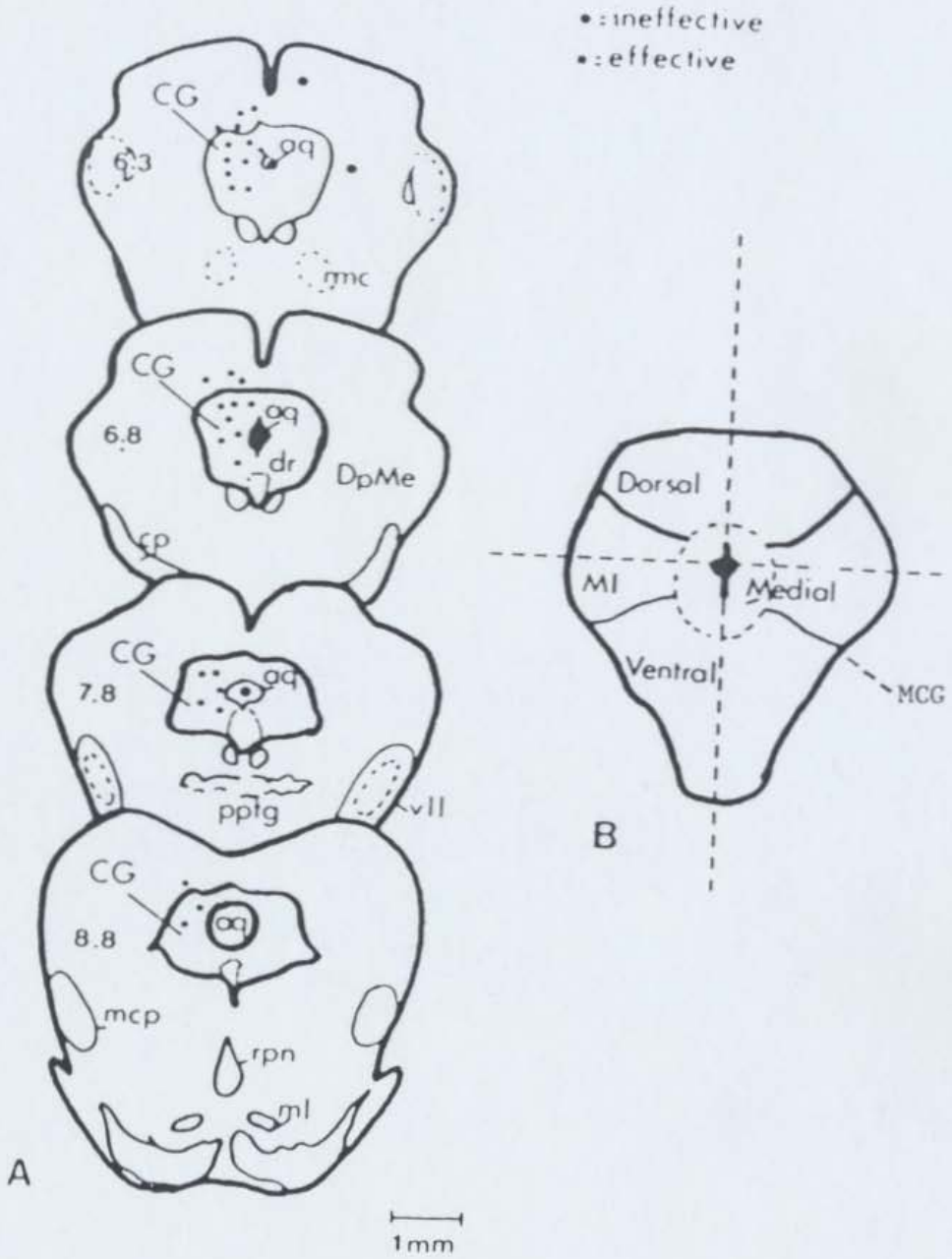

•: ineffective
•: effective
CG
6.3
aq
mc
CG
6.8
aq
dr
DpMe
cp
CG
7.8
aq
pptg
vll
CG
8.8
aq
mcp
rpn
ml
A
1mm
Dorsal
Ml
Medial
Ventral
MCG
B

Figure 2 Mean lordosis reflex score from -60 minutes (1 hour before infusion) through 300 minutes post infusion. Scores represent females' response to manual stimulation only. LHRH animals (50ng), n=12. = animals infused with saline (control), n=12. Lines represent ± standard errors of the mean.

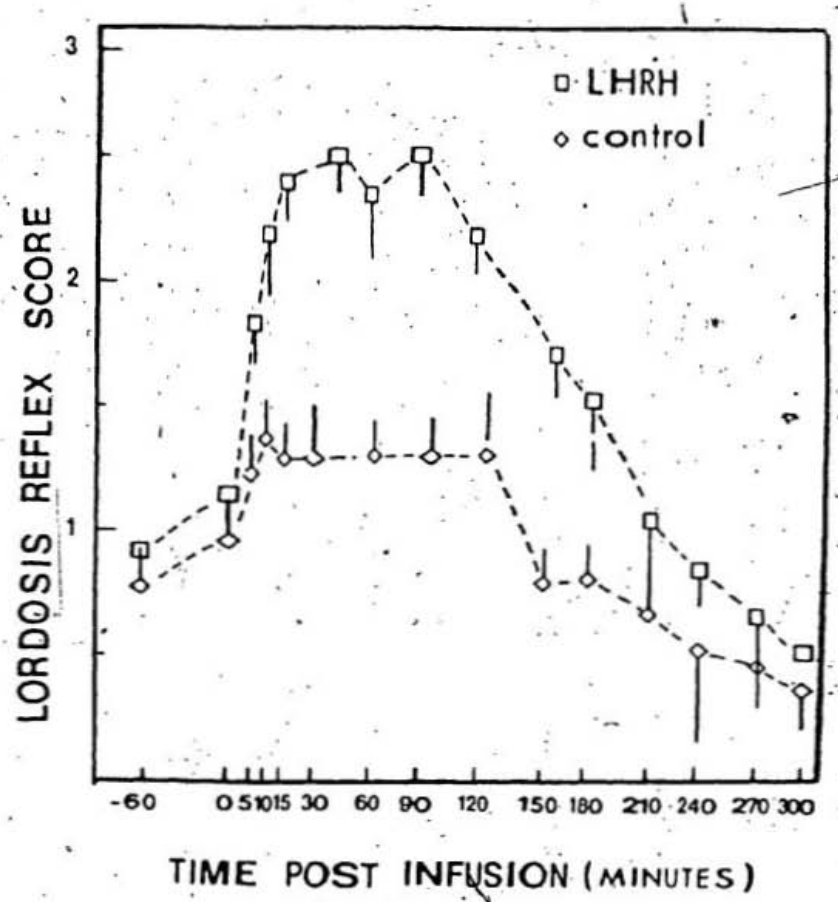

LHRH
control
LORDOSIS REFLEX SCORE
3
2
1
-60
0 5 10 15 30 60 90 120 150 180 210 240 270 300
TIME POST INFUSION (MINUTES)

Figure 3. Mean lordosis intensity score from -60 minutes (1 hour before infusion through 300 minutes post infusion). Scores represent females' response to stimulation from the male. LHRH animals (50ng), n=12. = control animals, n=12. Lines represent ± standard error of the mean.

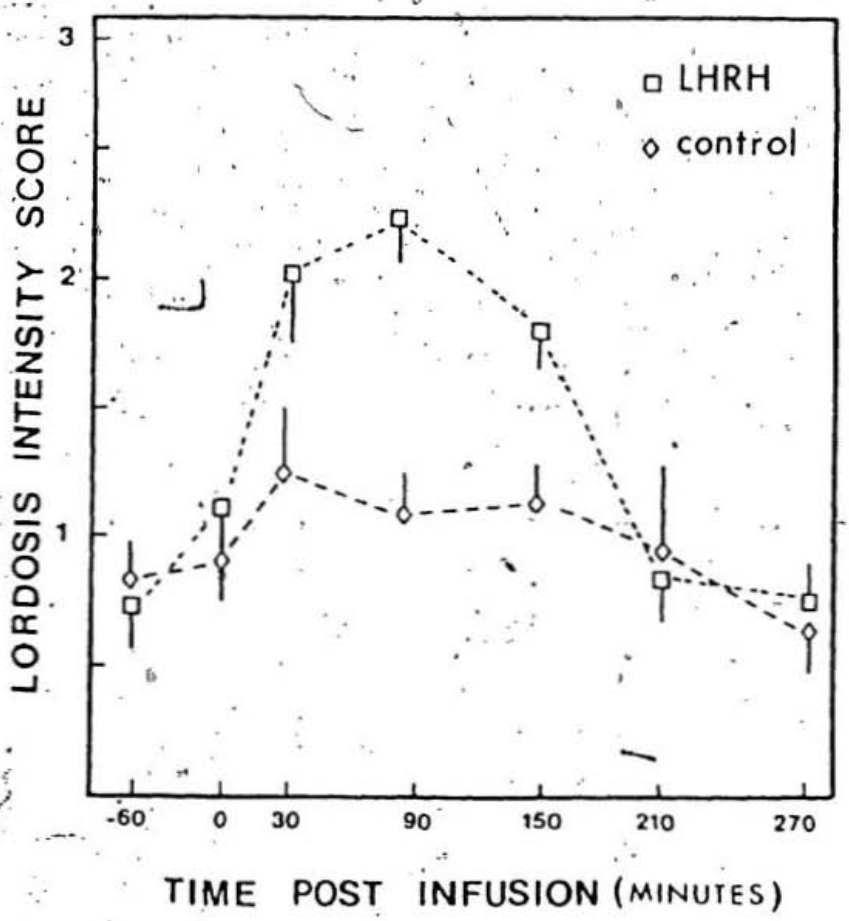

LORDOSIS INTENSITY SCORE
3
2
1
□ LHRH
◇ control
-60
0
30
90
150
210
270
TIME POST INFUSION (MINUTES)

TABLE 1

SUMMARY OF ANALYSIS OF VARIANCE (MANUAL STIMULATION)

LHRH INFUSIONS

Source	df	Sums of Squares	Mean square	F
Group	1	788.18	788.18	13.07 **
GxS	10	603.02	60.30	
Time	14	1579.02	112.81	33.69 **
TxS	140	468.76	3.35	
GxT	14	384.55	27.47	8.12**
GxTxS	140	473.26	3.38	

* = probability less than 0.05

** = probability less than 0.01

TABLE 2

SUMMARY OF ANALYSIS OF VARIANCE (MALE STIMULATION)

THRU INFUSIONS

Source	df	Sums of Squares	Mean Squares	F
Group	1	832.26	832.26	11.28 **
GxS	10	737.34	73.34	
Time	6	2130.99	355.16	30.68 **
TxS	60	694.59	11.58	
GxT	6	657.95	109.69	9.23 **
GxTxS	60	712.48	11.87	

* = probability less than 0.05

** = probability less than 0.01

180 minutes post infusion in the manual tests, and at 30, 90, and 150 minutes with the male. As figure 4 reveals, in addition to a peptide-induced facilitation of lordosis, a smaller facilitation was observed following infusion of the saline vehicle ($t=4.49$, $p<0.05$). This can also be seen in figures 2 and 3.

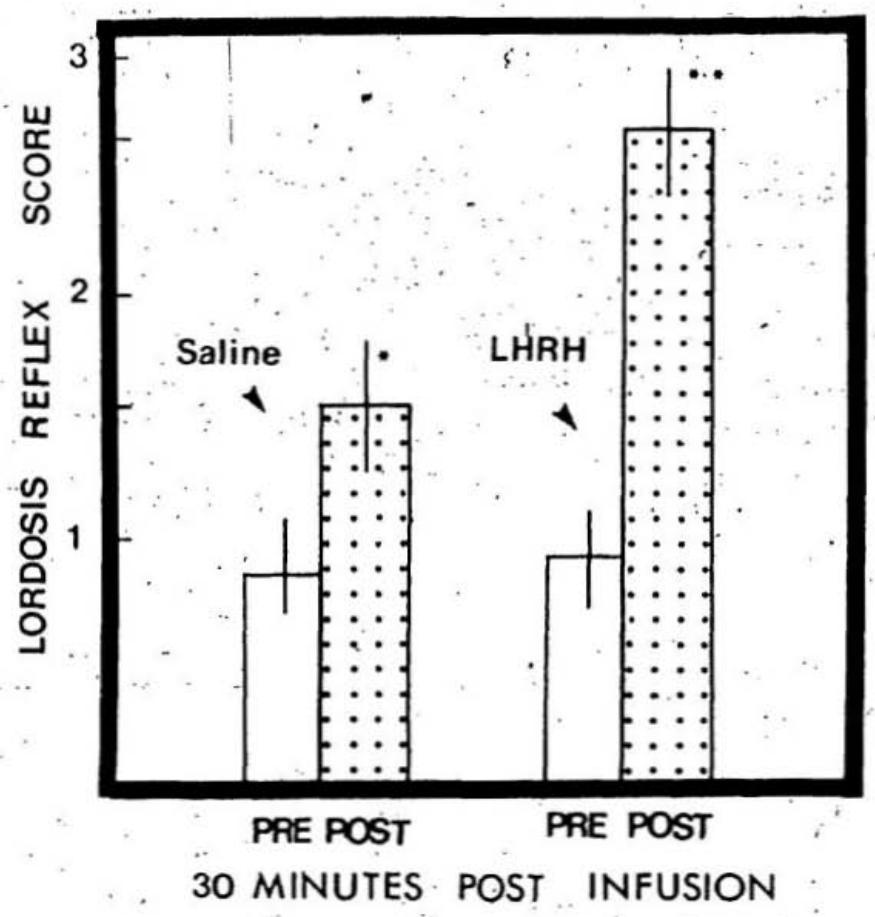
SUBSTANCE P

Originally, a completely counterbalanced design was desired, with all animals receiving the three doses of substance P. However this was not possible, because some animals could not be tested repeatedly, due to the separation of the guide cannula assembly from the skull. Thus the majority of animals did not receive all three infusions. Consequently, different infusion histories were treated as different experimental designs for the purpose of the analysis. Design 1: includes animals which received only one infusion of a peptide, hence a between group design; Design 2: animals which received all three doses of substance P, a repeated measures design; Design 3: animals that only received two doses of substance P, a repeated measures design.

DESIGN 1

Because of the differences between the

Figure 4 The effects of midbrain infusions of 50ng of LHRH on the lordosis reflex score at 30 minutes post infusion. Note the small increase in the control group. Lines represent ± standard error of the mean. * = $p<0.05$, ** = $p<0.01$


LORDOSIS REFLEX SCORE
3
2
1
Saline
LHRH
PRE POST
PRE POST
30 MINUTES POST INFUSION

number of animals in each group, i.e. 50 ng, n=4; 500 ng, n=2; 1 ug, n=6; a test of disproportionality was done before any further analysis was performed. The results of a Chi square on disproportionality revealed that the different groups were not disproportional (X = 2.01, $p<0.05$). Therefore, the data were analysed using an unweighted means analysis. Results of the analysis revealed a significant difference between the 50 ng, 500 ng, and the lug dose of substance, P versus the control group in the manual test (F=25.16, p 0.01; table 3), and in response to the male (F=5.69, $p<0.05$; table 4). As can be seen from figure 5, infusions of substance P produced a prompt facilitation of lordosis in response to manual stimulation that was evident as early as five minutes post infusion, and for as long as three and one half hours with both the 1 ug and 500 ng doses. As figure 6 illustrates, substance P also produced a facilitation of lordosis in response to the male which was evident at 30 minutes through 150 minutes post infusion with the lug dose. Substance P did not facilitate lordosis in response to male or manual stimulation in a dose-related manner (F=0.26, $p<0.05$; F=0.63 $p<$ 0.05 respectively, tables 3 and 4). However, a

Figure 5 Is a comparison of the mean lordosis reflex scores in animals that received one of the three doses of substance P (design 1). Facilitation can be seen as early as five minutes post infusion with all three doses. ▲ = 1ug, n=6; = 500ng, n=2; ◆ = 50ng, n=4. Lines represent + standard errors of the mean.

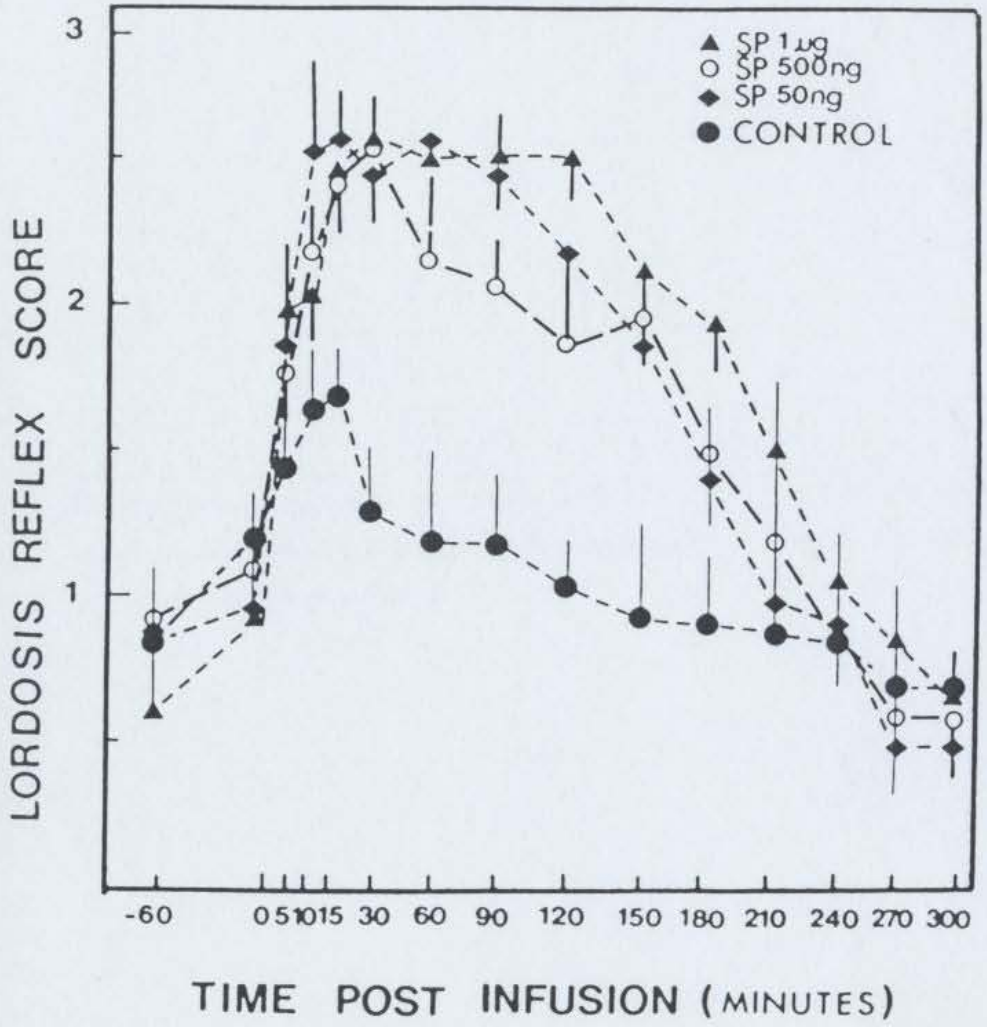

SP 1 µg
SP 500 ng
SP 50 ng
CONTROL
LORDOSIS REFLEX SCORE
3
2
1
-60
0 5 10 15 30 60 90 120 150 180 210 240 270 300
TIME POST INFUSION (MINUTES)

Figure 6 A comparision of the mean lordosis intensity score in animals that received one of the three doses of substance P (design 1).

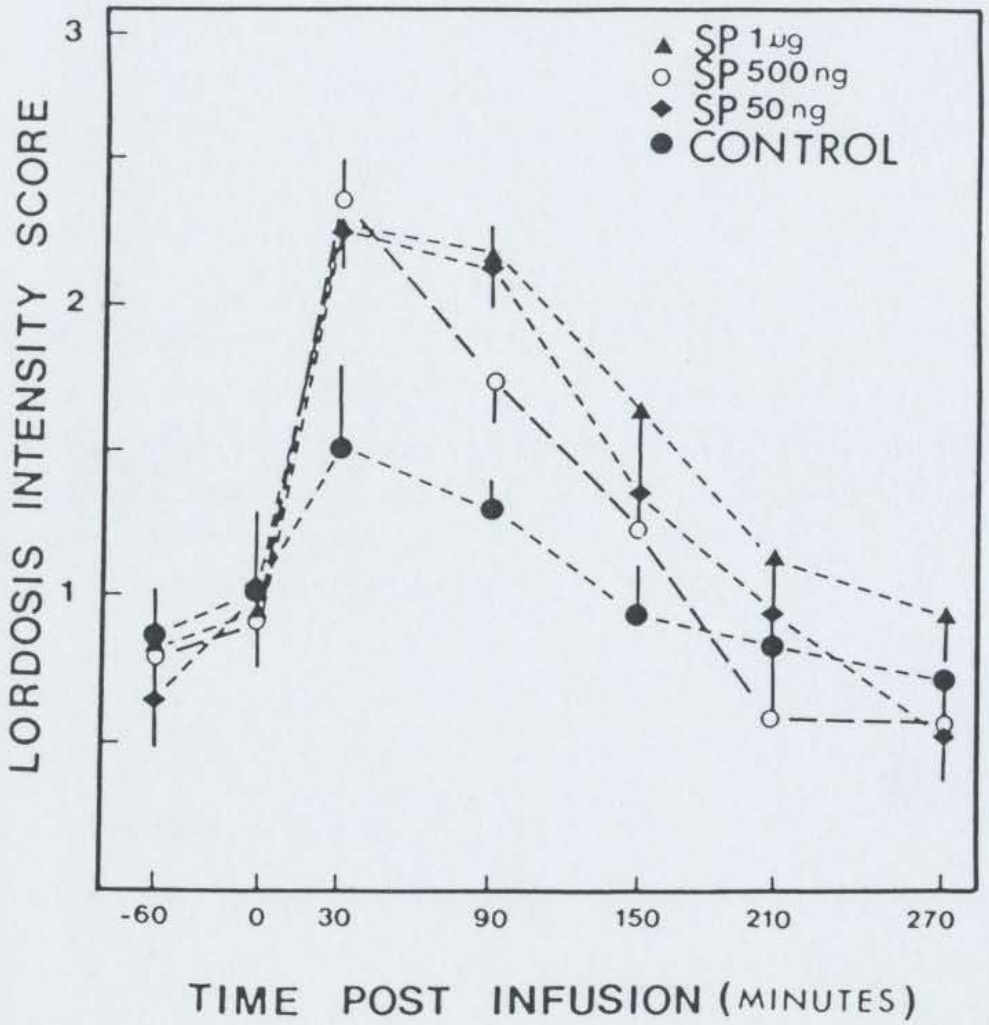

SP 1 µg
SP 500 ng
SP 50 ng
CONTROL
LORDOSIS INTENSITY SCORE
3
2
1
-60
0
30
90
150
210
270
TIME POST INFUSION (MINUTES)

TABLE 1

SUBSTANCE P INFUSIONS, DESIGN 1, BETWEEN GROUPS.
SUMMARY OF THE ANALYSIS OF VARIANCE. Manual stimulation.

Source	df	Sums of Squares	Mean Squares	F
BETWEEN GROUPS				
Group	1	855.63	855.63	25.16 **
Dose	2	4.27	2.13	00.63
GxD	2	44.98	22.49	00.66
S	18	611.92	33.99	
WITHIN GROUPS				
Time	14	1880.41	134.32	32.86 **
GxT	14	535.58	38.26	9.36 **
DxT	28	93.60	3.34	00.81
GxDxT	28	68.90	2.46	00.60
TxS	252	1029.90	4.09	

* = probability less than 0.05

** = probability less than 0.01

TABLE 4

SUBSTANCE P INFUSIONS, DESIGN 1, BETWEEN GROUPS.
SUMMARY OF THE ANALYSIS OF VARIANCE. Male stimulation.

Source	df	Sums of Squares	Mean Squares	F
BETWEEN GROUPS				
Group	1	317.63	317.63	5.69 *
Dose	2	29.01	14.51	0.26
GxD	2	39.54	19.77	0.35
S	18	1003.63	55.76	
WITHIN GROUPS				
Time	6	2880.37	480.06	34.81 **
GxT	6	526.75	87.79	6.36 **
DxT	12	82.23	6.85	00.49
GxDxT	12	80.21	6.68	00.48
TxS	108	1489.29	13.79	

* = probability less than 0.05

* * = probability less than 0.01

trend of increased facilitation of lordosis was observed with the largest dose of substance P yielding consistently higher scores from 90 minutes through 180 minutes post infusion (figure 5). In addition to a group effect, the analysis of variance revealed in both manual and male stimulation tests a time and a time by group interaction (tables 3 and 4). A comparison of substance P, 50 ng and LHRH 50 ng at 30 minutes post infusion with manual stimulation revealed a nonsignificant difference ($t=1.07$, $p<0.05$). This is illustrated in figs. 7 and 8.

DESIGN 2

In animals which received all three doses of substance P, each dose of the peptide led to an immediate facilitation of lordosis comparable to that observed in design 1 (figure 9). In addition, the magnitude of this effect was similar to that seen in design 1 in both the manual and male tests (figures 9 and 10). As tables 5 and 6 reveal, similar to design 1, different doses of substance P failed to produce differential responding in both the manual ($F= 1.66$, $p<0.05$) and male tests ($F= 3.34$, $p<0.05$). In order to establish whether the response to manual or male stimulation differed in animals that received a

Figure 7 A comparision of the effects of substance P (50ng) and LHRH (50ng) on the Lordosis reflex score. Substance P n = 13; LHRH, n = 11. Lines represent ± standard error of the mean.

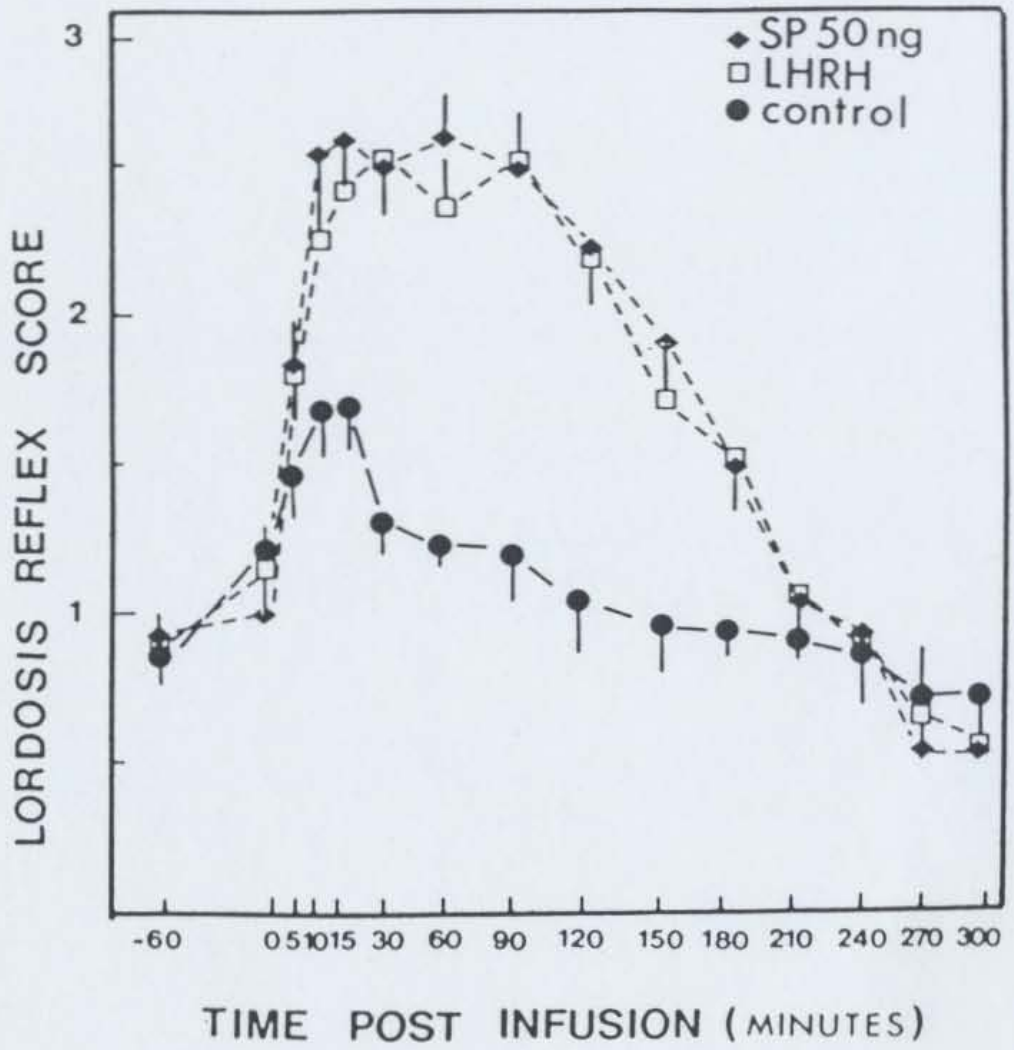

SP 50 ng
LHRH
control
LORDOSIS REFLEX SCORE
3
2
1
-60
0 5 10 15 30 60 90 120 150 180 210 240 270 300
TIME POST INFUSION (MINUTES)

Figure 8 Shows the same animals' responses to male stimulation

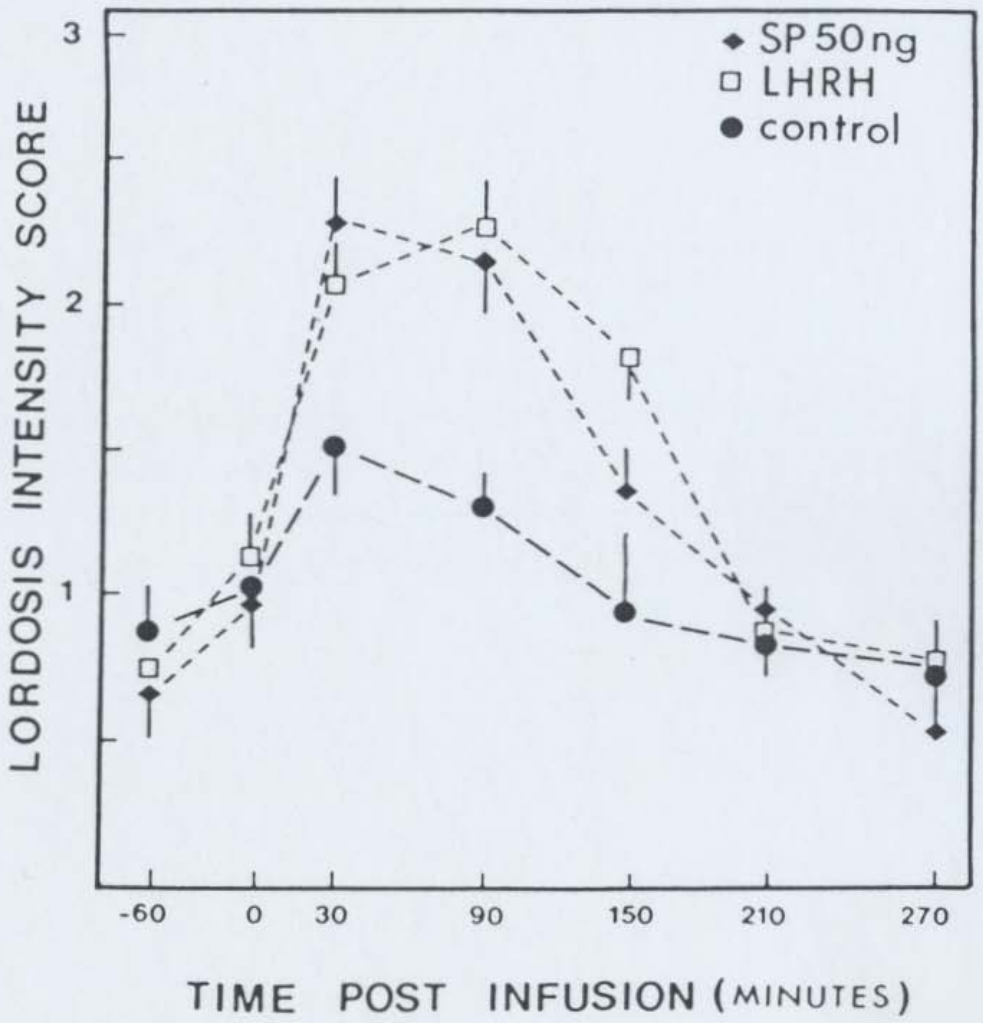

SP 50 ng
LHRH
control
LORDOSIS INTENSITY SCORE
3
2
1
-60
0
30
90
150
210
270
TIME POST INFUSION (MINUTES)

Figure 9 Mean lordosis reflex score after infusion of substance P in animals that received all three doses of substance P (n = 6). This corresponds to design 2. Note the increase in the control group (n = 6).

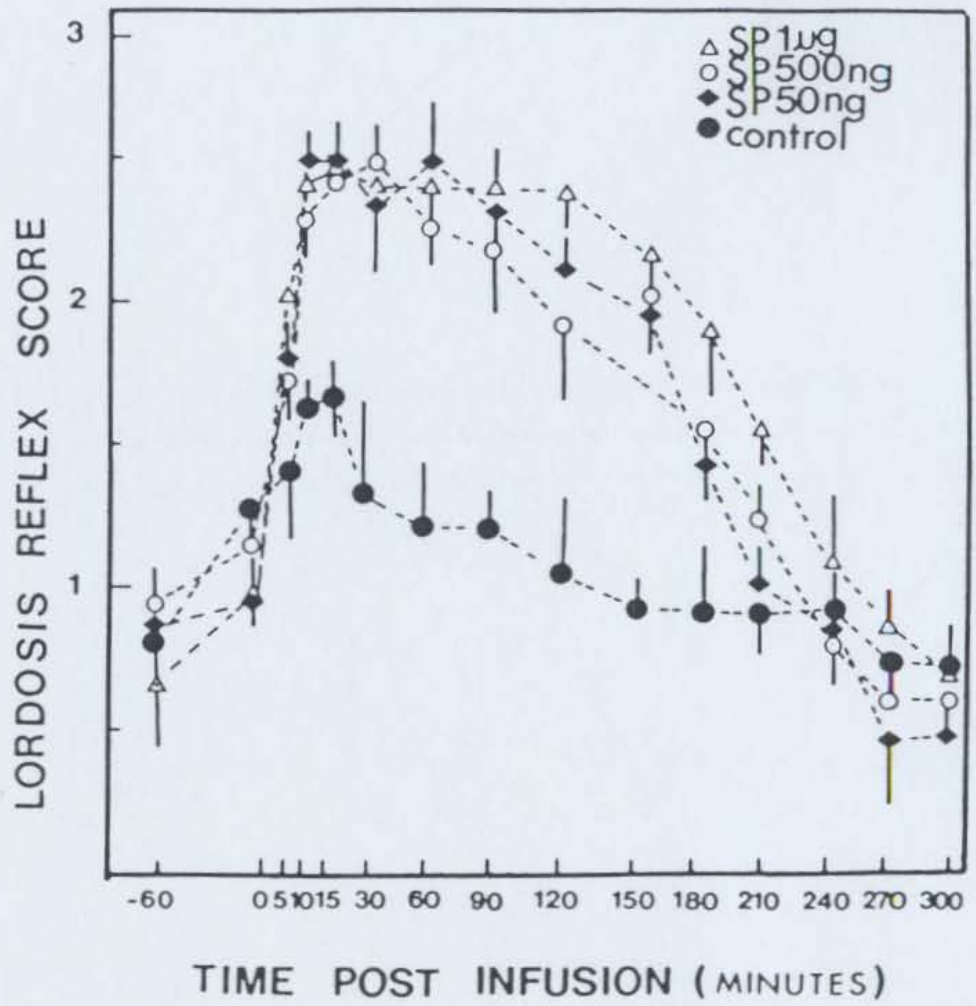

SP 1µg
SP 500ng
SP 50ng
control
LORDOSIS REFLEX SCORE
3
2
1
-60
0 5 10 15 30 60 90 120 150 180 210 240 270 300
TIME POST INFUSION (MINUTES)

Figure 10 Facilitation observed in response to the male in animals that received all three doses of substance P (design 2). Line represent ± standard errors of the mean.

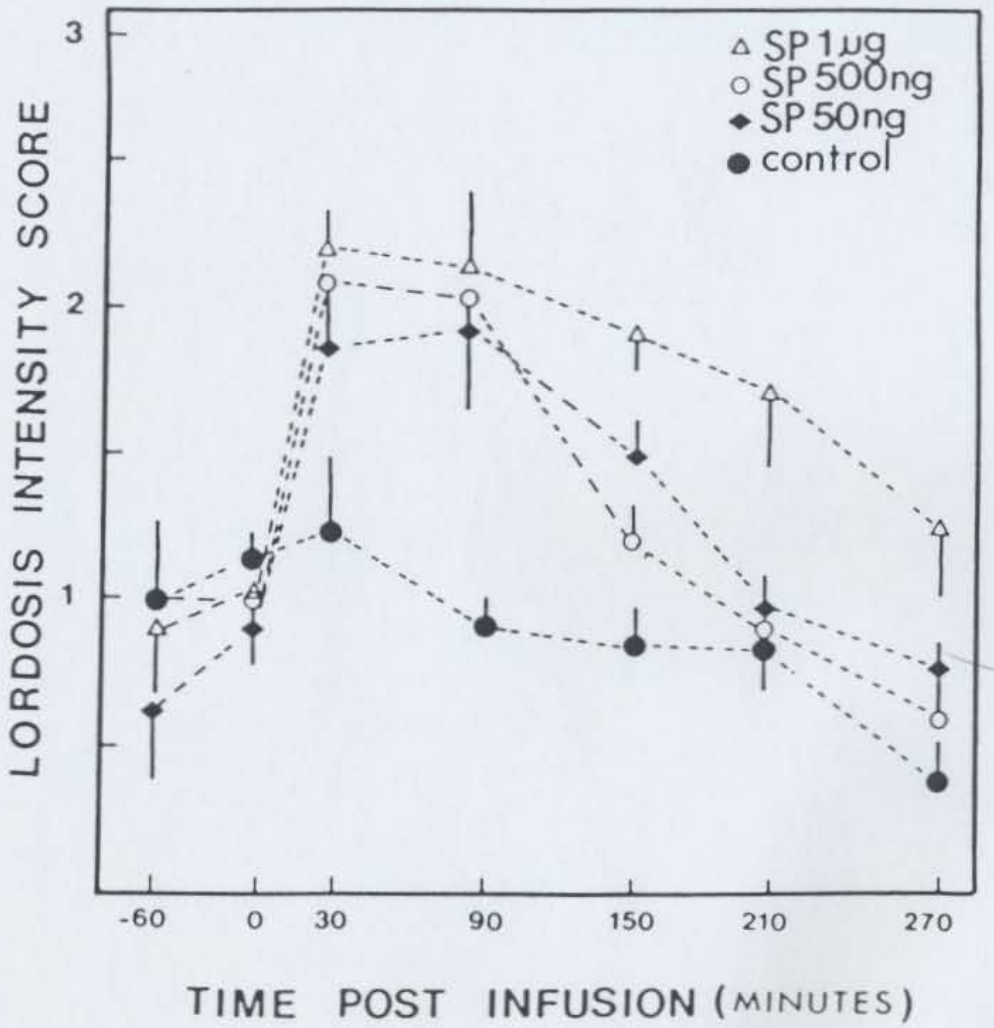

SP 1µg
SP 500ng
SP 50ng
control
LORDOSIS INTENSITY SCORE
3
2
1
-60
0
30
90
150
210
270
TIME POST INFUSION (MINUTES)

TABLE 5

SUBSTANCE P INFUSIONS, DESIGN 2, WITHIN GROUPS.

SUMMARY OF THE ANALYSIS OF VARIANCE. Manual stimulation.

Source	df	Sums of Squares	Mean Squares	F
BETWEEN GROUPS				
Group	1	1294.25	1294.25	47.81 **
S	10	270.69	27.07	
WITHIN GROUPS				
Dose	2	133.07	66.54	1.66
GxD	2	108.67	54.33	1.36
DxS	20	801.95	40.10	
Time	14	2189.49	156.39	37.28 **
GxT	14	419.69	29.97	7.14 **
TxS	140	587.30	4.19	
DxT	28	97.87	3.50	0.82
GxDxT	28	149.38	5.34	1.25
DxTxS	280	1199.05	4.28	

* = probability less than 0.05

** = probability less than 0.01

TABLE 6

SUBSTANCE P INFUSIONS, DESIGN 2, WITHIN GROUPS.
SUMMARY OF THE ANALYSIS OF VARIANCE. Male stimulation.

Source	df	Sums of Squares	Mean Squares	F.	
BETWEEN GROUPS					
Group	1	1334.92	1334.92	16.32	**
S	10	817.83	81.78		
WITHIN GROUPS					
Dose	2	292.68	146.34	3.34	
GxD	2	237.20	118.60	2.71	
DxS	20	876.51	43.83		
Time	6	2519.37	419.90	25.83	**
GxT	6	1226.58	204.43	12.58	**
TxS	60	975.29	16.25		
DxT	12	222.27	18.52	1.99	*
GxDxT	12	226.64	18.89	2.03	*
DxTxS	120	1114.69	9.30		

* = probability less than 0.05

** = probability less than 0.01

given dose of peptide on the first, second, or third administration, an analysis of variance on order was carried out. As can be seen from table 7, whether an animal received a particular dose of substance P on the first, second, or third administration did not significantly change its response to manual or male stimulation ($F = 0.024$, $p < 0.05$).

DESIGN 3

As shown in figure 11, in male tests, animals which received 500ng and 1ug of substance P showed a facilitation of lordosis at 30 minutes as compared to controls ($F = 15.45$, $p < 0.01$, table 8). In addition, as table 8 reveals, a dose-dependent facilitation of lordosis was observed. This can also be seen in figure 11. As can be seen from table 9 and figure 12, animals which received 500 and 50ng of substance P failed to differ significantly from controls ($F = 4.85$, $p < 0.05$). However a time and a group by time interaction were observed (table 9).

ANTISERUM

Infusions of undiluted antiserum (n=8), failed to significantly decrease lordosis scores in response to male stimulation ($F = 0.42$, $p < 0.05$; table 10, fig. 13). Although no overall

TABLE 7

SUMMARY OF THE ANALYSIS OF VARIANCE ON DOSE ORDER.

Source	df	Sums of Squares	Mean Squares	F
Dose	2	.537	.269	0.06
Order	4	.400	.100	0.02
DxO	8	1.062	.132	0.04
Within cell	6	13.00	4.333	

Figure 11 Mean lordosis intensity score in animals that received 1 ug (n=5), and 500 ng (n=5) of substance P (design 3). Lines represent ± standard errors of the mean.

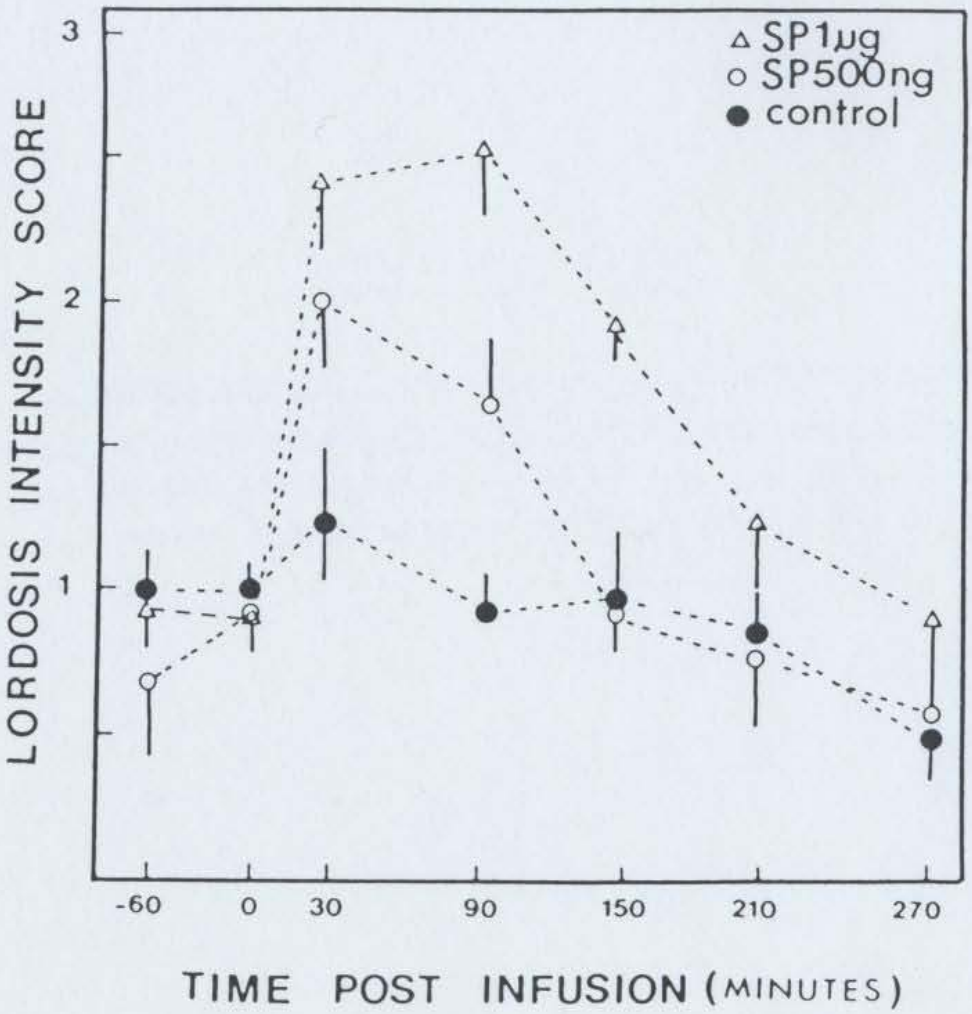

△ SP1µg
○ SP500ng
● control
LORDOSIS INTENSITY SCORE
3
2
1
-60
0
30
90
150
210
270
TIME POST INFUSION (MINUTES)

Figure 12 'Represents mean lordosis scores in animals that received 50 and 500ng of substance P (n = 4). Filled circles represent control group which received a vehicle infusion (n = 4). Lines represent ± standard errors of the mean.

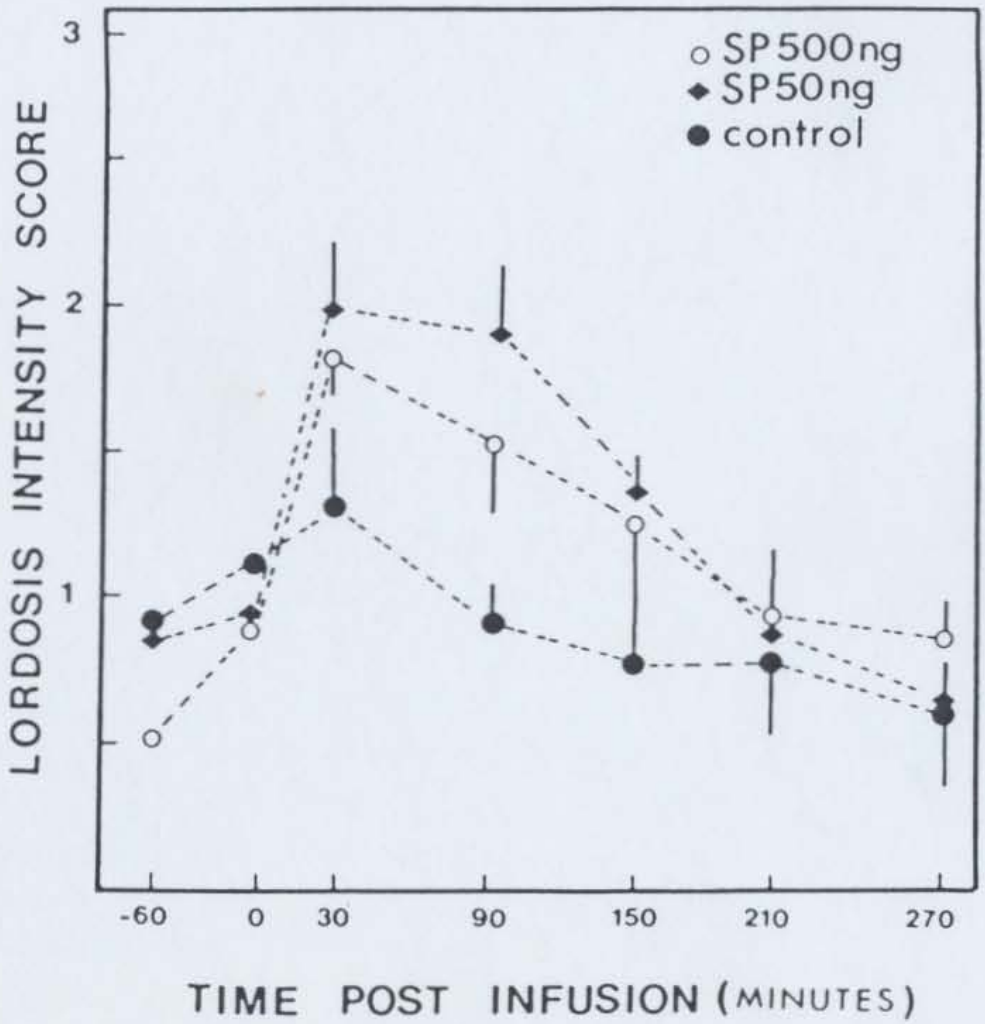

SP500ng
SP50ng
control
LORDOSIS INTENSITY SCORE
3
2
1
-60
0
30
90
150
210
270
TIME POST INFUSION (MINUTES)

TABLE 8

SUBSTANCE P INFUSIONS, DESIGN 4, WITHIN GROUPS, 500ng and 1 ug.

SUMMARY OF THE ANALYSIS OF VARIANCE. Male stimulation.

Source	df	Sums of Squares	Mean Squares	F	
BETWEEN GROUPS					
Group	1	584.26	584.26	15.45	**
S	8	302.34	37.79		
WITHIN GROUPS					
Dose	1	274.40	274.40	6.78	*
GxD	1	206.43	206.43	5.10	
DxS	8	323.31	40.41		
Time	6	1803.67	300.61	20.98	**
GxT	6	785.44	130.91	9.14	
GxS	48	687.45	14.32		
DxT	6	111.90	18.65	2.21	
GxDxT	6	107.67	17.96	2.13	
DxTxS	48	403.29	8.41		

* = probability less than 0.05

** = probability less than 0.01

TABLE 9

SUBSTANCE P INFUSION, DESIGN 3, WITHIN GROUPS, 50 and 500 ng.
SUMMARY OF THE ANALYSIS OF VARIANCE, Male stimulation.

Source	df	Sums of Squares	Mean Squares	F
BETWEEN GROUPS				
Group	1	211.75	211.75	4.85
S	6	261.82	43.64	
WITHIN GROUPS				
Dose	1	0.32	0.32	0.59
GxD	1	17.26	17.26	0.31
DxS	6	326.25	54.38	
Time	6	1075.75	179.29	16.38 **
GxT	6	533.75	88.96	8.12 *
TxS	36	393.93	10.94	
DxT	6	105.18	17.53	1.26
GxDxT	6	143.96	23.99	1.73
DxTxS	36	498.00	13.83	

* = probability less than 0.05

** = probability less than 0.01

Figure 13 Illustrates mean lordosis intensity score in animals that received a midbrain central gray infusion of antiserum to substance P (n=8), or normal rabbit serum(n=8). Lines represent ± standard errors of the mean.

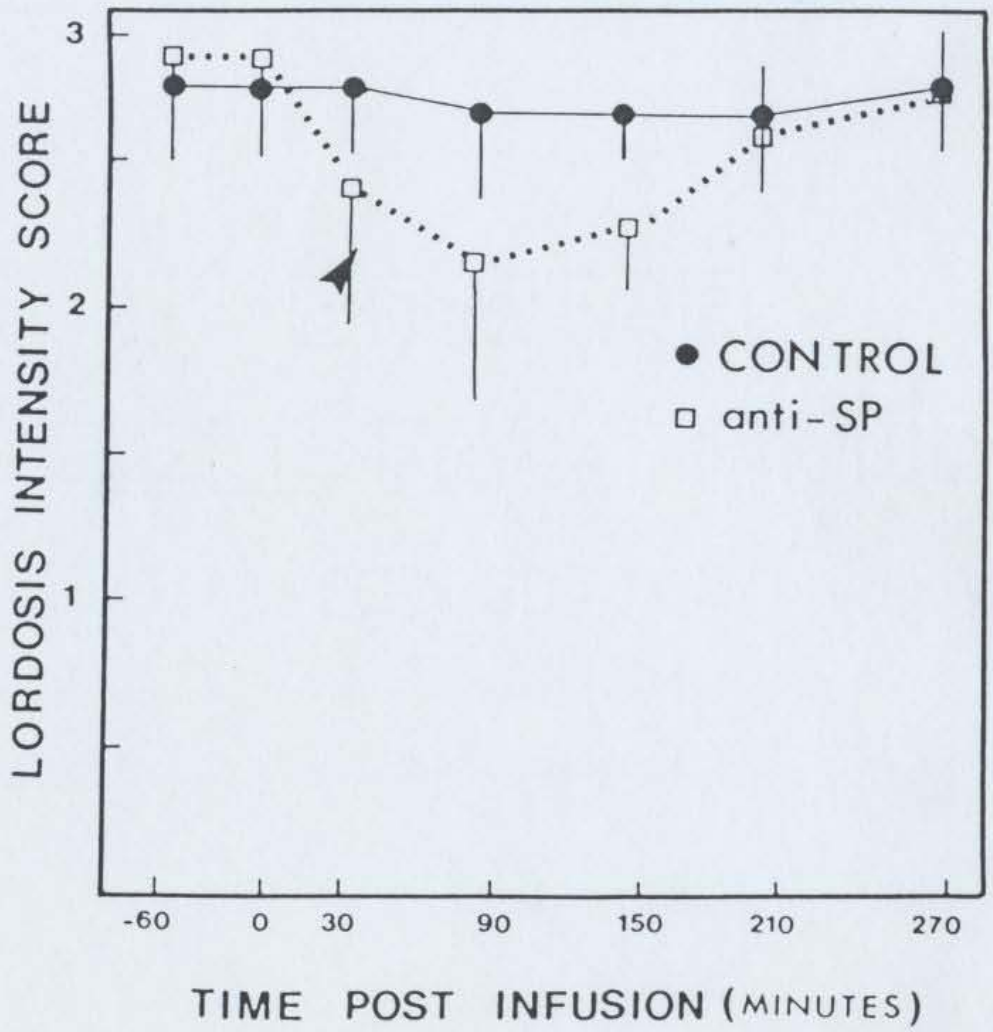

LORDOSIS INTENSITY SCORE
3
2
1
● CONTROL
□ anti-SP
-60
0
30
90
150
210
270
TIME POST INFUSION (MINUTES)

TABLE 10

SUMMARY OF THE ANALYSIS OF VARIANCE FOR ANTI-SUBSTANCE P.
Male stimulation.

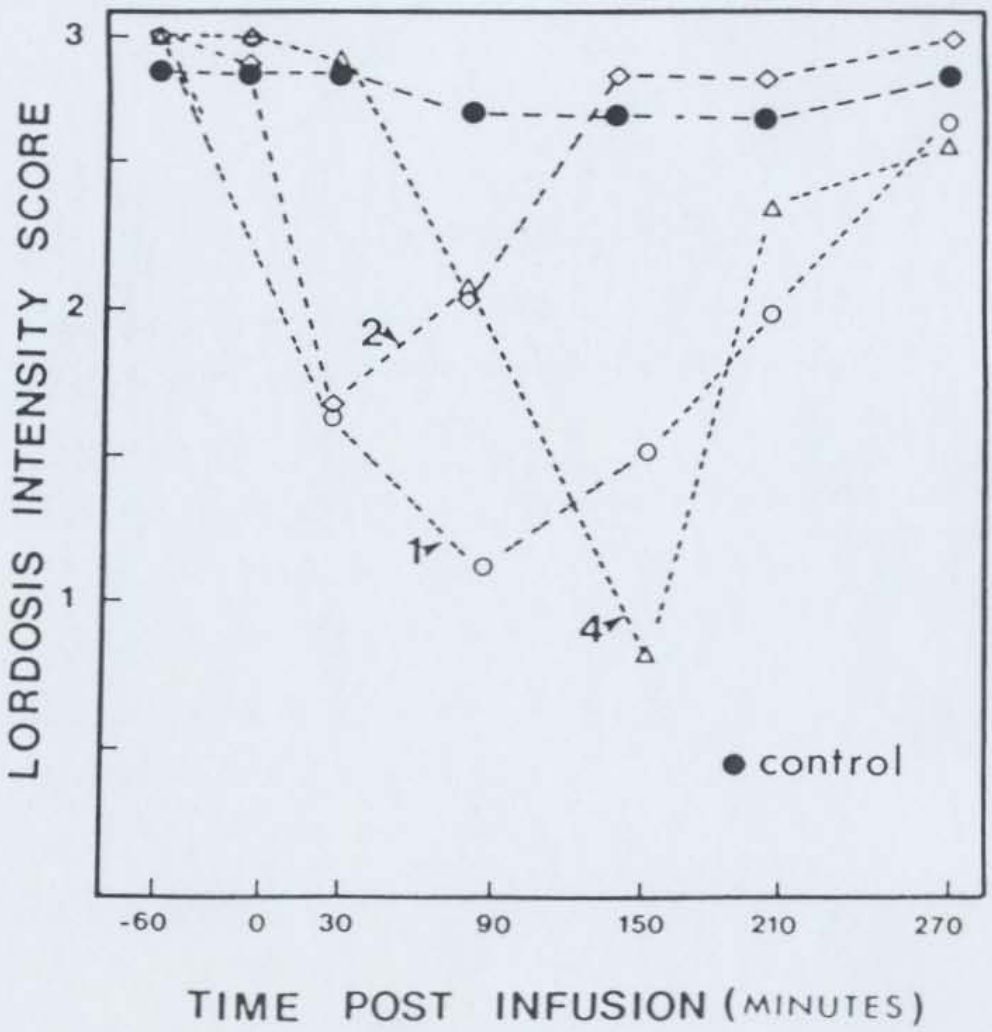
Source	df	Sums of Squares	Mean Squares	F
Group	1	25.08	25.08	0.42
GxS	7	412.70	58.96	
Time	6	225.30	37.55	3.55 *
TxS	42	443.55	10.56	
GxT	6	142.48	23.74	2.64 *
GxTxS	42	377.21	8.98	

* = probability less than 0.05

** = probability less than 0.01

significant effect was observed , the substance P antiserum was not without effect, in that three out of the eight animals displayed a lordosis deficit when tested with the male while none of the controls showed any such deficits (figure 14). Table 10 also shows that although no group effect was observed, there was a significant time effect and a group by time interaction ($F = 3.55$, $p < 0.05$; $F = 2.64$, $p < 0.05$).

Figure 14 Shows the disruptive effect of midbrain infusions of anti-SP on the mean lordosis intensity score for three individual females (no.'s 1, 2, and 4) .


LORDOSIS INTENSITY SCORE
3
2
1
2
1
4
● control
-60
0
30
90
150
210
270
TIME POST INFUSION (MINUTES)

DISCUSSION

Infusions of 50ng of LHRH bilaterally into the midbrain central gray were effective in augmenting lordosis in response to male and manual stimulation in ovariectomized estrogen-treated female rats. Following midbrain infusions of LHRH, the lordosis reflex score (manual stimulation) increased within 5 minutes, reached a peak at 90 minutes, and returned to baseline levels by 180 minutes post infusion. In response to the male, LHRH resulted in significant facilitation of lordosis at 30, 90, and 150 minutes post infusion. These data lend further support to the role of LHRH in the regulation of lordosis in female rats (Sakuma and Pfaff, 1983). In contrast to results published by Sakuma and Pfaff (1983) however, was the finding that infusions of LHRH through cannulae that were located more ventrally in the midbrain central gray were also effective in facilitating lordosis. Riskind and Moss (1979) have also found that LHRH infusions into the ventral central gray of the midbrain facilitated lordosis.

In addition to the facilitation observed after LHRH infusions, infusion of the different doses of substance P all led to a prompt facilitation of lordosis in response to male and

manual stimulation. This facilitation was also evident within the first five minutes post infusion, and depending on the dose, lasted for as long as two and a half hours. Although three different doses of substance P were used (50 ng, 500 ng, and 1 ug) , a dose-response relation could not be demonstrated statistically, despite an apparent trend. For example, the scores of animals that received 1 ug of substance P did not differ overall statistically from the scores of animals receiving the other two doses. However, 1 ug did yield consistently higher lordosis scores, particularly during the latter half of the testing sessions in both male and manual tests (figs. 4 and 5). This was not restricted to animals that received the different doses of substance P independently (design 1), but was also apparent in animals that were given all three (design 2, figs 8 and 9) or only two doses of substance P (design 3, Fig. 11).

The results of these experiments suggest that, in addition to LHRH, substance P, plays a role in the modulation of receptivity in female rats.

What Is the Role of Substance P ?

a/ The VMH.

The ventromedial nucleus of the hypothalamus (VMH), sends projections to the midbrain central gray via a medial projection, periventricular, and a lateral one which first projects antero-laterally in the area of the supraoptic commissures and then descends to the midbrain (Krieger et al., 1979). The integrity of these pathways seems important in that if the lateral pathway is disrupted, deficits in lordosis occur in both hamsters and rats (Malsbury and Daood, 1978; Manogue et al., 1980). In addition, although perhaps not as important, a disruption of the medial pathway results in temporary deficits in lordotic responsiveness (Manoque et al., 1980). Moreover, inhibition of axoplasmic flow from the VMH to the MCG by the use of colchicine produces deficits in lordosis in female rats (Harlan et al., 1982). Although lending further support to the importance of VMH efferents to the midbrain central gray, the latter study must be interpreted with caution as intrahypothalamic VMH administration of colchicine does not selectively reduce axoplasmic flow only in the aforementioned efferent path.

The VMH contains numerous substance P-like immunoreactive cell bodies (Ljungdahl et al., 1978), as well as receptors (Quirion et al., 1983).

In addition, it has been shown that substance P can be released from hypothalamic terminals (Iversen, Jessell, and Kanazawa, 1979). In that study they report that endogenous substance P-like immunoreactivity could be released from hypothalamic tissue in vitro, in response to a depolarizing stimulus. In addition, substance P has also been shown to influence midbrain central gray neurons. For example, in a study done by Del Rio, Naranjo, Yang, and Costa (1983), substance P released met-enkephalin from midbrain central gray slices in a calcium dependent fashion. Although the origin of midbrain central gray substance P has not been determined, it is reasonable to postulate that VMH efferents transport substance P to the midbrain central gray where it is released from nerve terminals to affect midbrain central gray neurons.

b/ Endogenous Opiates, Analgesia, and Receptivity.

The involvement of endogenous opiates in female sexual behavior has been documented. For example, Sirinathsinghji et al., (1983), reported that when naloxone was infused into the midbrain central gray, an immediate facilitation of lordosis was observed. Furthermore, in that same study they were able to demonstrate that central gray

infusions of B-endorphin suppressed lordosis, and that infusion of met-enkephalin had no effect. This suppression could be overcome by pretreatment with an intraperitoneal injection of naloxone, or by subsequent infusions of LHRH into the midbrain central gray through the same cannula. Based on these observations, they concluded that B-endorphin controls sexual receptivity in female rats by presynaptic inhibition of LHRH release. However since B-endorphin has been reported to inhibit substance P release from the rat trigeminal nucleus (Jessell and Iversen, 1977), the facilitation observed after naloxone treatment may be the result of a disinhibition of substance P release. Moreover, since substance P has been shown to release met-enkephalin from midbrain central gray in vitro (Del Rio et al., 1983), during the unreceptive phase of the rat's estrous cycle, B-endorphin by inhibiting substance P release could keep met-enkephalin levels low in the midbrain central gray (an area known to be involved in analgesia), thereby making mounting attempts by the male intolerable to the female during the unreceptive phase. The corollary of this would be that during the receptive phase, when estrogen levels are comparatively high, increased VMH

neuronal activity could result in greater release of substance P in the midbrain central gray, resulting in local met-enkephalin release and making the mounting and multiple intromissions by the male tolerable. Substance P when infused into the midbrain central gray has been shown to induce analgesia in rats (Malick and Goldstein, 1978; Mohrland and Gebhart, 1979). In the Malick and Goldstein study (1978) they reported a dose dependent substance P-induced analgesia, which was naloxone reversible. More interestingly, closer examination of their data reveals similarities between the analgesia observed in their study and the facilitation of lordosis obtained in this one. For example, substance P infusions into the midbrain central gray led to a prompt facilitation of lordosis that was evident as early as five minutes post infusion, and in some animals was observed to peak between 5 and 15 minutes (although overall the peak responsiveness occurred later). In the Malick and Goldstein study the peak analgesic effect also occurred within this time frame. Furthermore, Naranjo, Fernandez-Tome, and Del Rio (1982) have shown that analgesia induced by intraventricular infusion of substance P can be blocked by a met-enkephalin antiserum. In

that study they also reported a dose-dependent substance P-induced analgesia. This could be completely eliminated, however, if substance P and an antiserum against met-enkephalin were administered at the same time. The analgesic effect of substance P could not be blocked, however, with a B-endorphin antiserum, suggesting that the substance P-induced analgesia was mediated by met-enkephalin release, and not B-endorphin. Although the above hypothesis concerning the interaction of Met-enkephalin and substance P for lordosis is an interesting one, there is one major inconsistency with it. For example, if met-enkephalin is involved in lordosis control, why doesn't infusion of met-enkephalin into the central gray potentiate lordosis ? Perhaps the best explanation for this is as follows: although met-enkephalin when infused alone into the MCG does not facilitate lordosis, that does not necessarily mean it is not important. Met- enkephalin could interact with substance P to facilitate lordosis. For example, met-enkephalin could during the receptive phase of the estrous cycle produce analgesia which enables the male to approach the female, then, after tactile stimulation from the male substance P could then facilitate lordotic

responsiveness. Therefore, if one only infused met-enkephalin alone one would not be surprised if facilitation of lordosis did not occur. One way to test this hypothesis would be to first infuse a met-enkephalin antiserum followed by substance P and compare these animals to animals that just received substance P infusions. In fact, this project is being considered.

c/ The VMH, Midbrain Central Gray, and the Estrous Cycle of the Female.

Estrogen levels fluctuate during the estrous cycle of the female rat. Futhermore there is some evidence that substance P neurons are influenced by fluctuations in gonadal steroids as Antonowicz, Jakubowska-Naziembo, Cannon, and Powell (1982) have found that substance P levels within the median eminence fluctuate with the estrous cycle of the female rat. Estrogen increases VMH cell activity, and this rise possibly results in concomitant release of substance P from the terminals of VMH neurons in the midbrain central gray. Indeed, Morrell and Pfaff, (1982), have reported recently that when using the combined steroid radiography-retrograde tracing method, they were able to identify a substantial number of estrogen concentrating cells located in the VMH

which project directly to the midbrain central gray. Moreover, they found that most of those cells were located in the ventral lateral segment of the VMH, the area of the VMH with the highest concentration of substance P immunoreactive cells (Ljungdahl et al., 1978; and personal observation). Therefore, during the estrous cycle of the female rat when estrogen levels are high, increased VMH neuronal activity could ultimately result in a heightened sensitivity of central gray neurons. Sakuma and Pfaff (1979a), found that electrical stimulation of the midbrain central gray facilitated lordosis in estrogen-primed female rats. They reported that this facilitation was immediate. Substance P infusion into the central gray also produced an immediate facilitation in the present study. In fact the latency and duration of this facilitation closely resembles the increase in lordosis scores observed by Sakuma and Pfaff. Consequently, electrical stimulation of the midbrain central gray could result in release of endogenous substance P which could modulate central gray neurons involved in the control of lordotic responsiveness. Substance P could then theoretically facilitate lordotic responsiveness directly, by post-synaptic action and subsequent

release of another substance; perhaps an endogenous opiate (central gray neurons e.g. met-enkephalin cells, Del Rio et al., 1983), or indirectly by presynaptic modulation of LHRH and/or prolactin, both of which have been recently shown to facilitate lordosis when infused into the midbrain central gray (Sakuma and Pfaff, 1983 ; Harlan et al., 1983b).

Does substance P play a role in mediating vaginal-induced analgesia ?

Probing the vaginal cervix with a glass rod induces immobilization and abolishes the leg withdrawal reflex to foot pinch in female rats (Komisaruk and Diakow, 1973). In fact, vaginal stimulation is more potent than morphine in suppressing a nociceptive response in rats (Komisaruk, Ciofalo, and Latranyi, 1976), and recently has been shown to induce analgesia in humans (Whipple, M.Ed, and Komisaruk, 1984). This cervical-induced analgesia produced in rats is intensified after estrogen treatment (Crowley, Jacobs, Rodriguez-Sierra, and Komisaruk, 1976). Since it has already been mentioned that substance P levels fluctuate with the estrous cycle of the female rat, perhaps cervical stimulation activates substance P fibers which mediate met-enkephalin

release thereby inducing analgesia and immobilization, a necessary component of mating. This would only lead to a greater facilitation of lordosis, and not be responsible for its initiation.

d/ Nucleus Gigantocellularis

The midbrain central gray sends very few direct projections to the spinal cord (although it receives numerous projections from the cord). It does, however, send extensive projections to the nucleus gigantocellularis of the medulla, which has reciprocal connections with the cord and midbrain central gray. In fact, nucleus gigantocellularis neurons respond to lordosis-relevant tactile stimuli (Kow, Gyill, and Pfaff, 1978), and lesions of the nucleus gigantocellularis produce deficits in lordosis in tests with manual stimulation (Modianos and Pfaff, 1979). Since substance P cell bodies and receptors have been found in this nucleus (Ljungdahl et al., 1978; Quirion et al., 1982), facilitation of lordosis by midbrain central gray infusions of substance P could be acting via the nucleus gigantocellularis efferents to the spinal cord. Therefore, during the receptive phase of the rat's estrous cycle when estrogen levels are high, somatosensory stimulation

from the male could result in substance P release on midbrain central gray neurons as well as in the nucleus gigantocellularis, thereby producing lordosis and possibly analgesia which makes multiple intromissions possible.

e/ Necessity of Midbrain Release of Substance P for Lordosis.

Although the failure of the antiserum to produce a decrement in lordosis was disappointing, subsequent re-evaluation of the circumstances has revealed a number of possible explanations. Firstly, animals that were in the antiserum group had had at least two previous infusions of either substance P or vehicle. Consequently, cellular damage around the cannula tip could have resulted in the high variability obtained. Secondly, the behavioral result of lordosis disruption might depend on blocking substance P release bilaterally in a large area of central gray, as substance P terminals appear to be widespread throughout the central gray. As a result, facilitation with substance P might be easier to produce than disruption with a substance P antiserum. Thirdly, the technology of using antiserum raised against a particular peptide for behavioral evaluations is, at best, a relatively new path. Therefore,

negative results (as well as positive ones), should be interpreted with extreme caution. Lastly, although statistical significance was not obtained, a trend of decreased lordosis scores after administration of the antiserum was apparent. Most importantly, of the three animals which showed a deficit after antiserum infusion, two had previously shown a facilitation of lordosis when infused with substance P.

Perhaps the most important point to make concerning the necessity of substance P for lordosis is the difficulty with extrapolations of behavior and the subsequent localization of action of the neurochemical substance. For example, diffusion does occur after peptide infusion into the brain. Since, in this study, the cerebral aqueduct was on several occasions in close proximity to infusion sites, diffusion into the ventricle could certainly have occurred. However, in some instances, when both cannulae were inadvertently placed in the cerebral aqueduct, subsequent testing revealed very low lordosis scores. Consequently, these animals could be used as unintended controls.

In conclusion, infusions of both substance P and LHRH into the midbrain central gray of female

rats resulted in a prompt and long lasting facilitation of lordosis in response to male and manual stimulation. The facilitation obtained with substance P closely resembled that of LHRH in latency and duration. It did not, however, resemble the facilitation observed after prolactin infusions into the midbrain central gray in the Harlan et. al., (1983) study; the onset was slower but the duration longer. Infusions of antiserum raised against substance P failed to significantly reduce lordosis scores, although a trend was evident. Whether substance P modulates receptivity via release from VMH efferents to the midbrain central gray remains unclear. In addition, whether substance P acts alone or interacts with other peptides to produce this facilitation remains to be determined.

References

Amoss,M. Blackwell,R. and Guillemin,R. Stimulation of ovulation in the rabbit triggered by synthetic LHRF. Journal of Clinical Endocrinology Metabolism. 34, 434-436, 1972

Antonowicz, U., Jakubowski-Naziemblo, B., Cannon, D., and Powell, D. Immunoreactive substance P content in the median eminence and pituitary gland during oestrus, dioestrus, and after anterior hypothalamic deafferentation. Endokrinologie. 79, 25-34, 1982.

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

Barry, J., Dubois, M.P. Immunoreactive LHRF neurosecretory pathways in Mammals. Acta Anatomy. 94, 497-503, 1976.

Beach, F.A. Hormones and Behavior. In W.C. Young (Ed) Sex and Internal Secretions, Vol. 2 Baltimore, Williams and Wilkins 1948.

Beach, F.A. Sexual atractivity, proceptivity, and receptivity in female Mammals. Hormones and Behavior. 7 105-138, 1976.

Bermant, G., Davidson, J. Biological Bases of Sexual Behavior. Harper and Row Publishers, New York, Evanson, San Francisco, London, 1974.

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

Carret, H., Asch, G., Aron, C. New facts concerning the role played by the ventromedial nucleuss in the control of estrous cycle duration and sexual receptivity in the rat. Neuroendocrinology. 13, 129-138, 1973.

Chan, A., Dudley, C.A., and Moss, R.C. Action of Prolactin, Dopamine, and LHRH on Ventromedial Hypothalamic Neurons as a function of Ovarian Hormones. Neuroendocrinology. 36, 397-403, 1983.

Clark, R.C., Maclusky, N.J., Parsons, B., Naftolin, F. Effects of estrogen deprivation and brain estrogen and progesterone levels and the activation of female sexual behavior. Hormones and Behavior. 15 289-298, 1981.

Clayton, C.J., Hoffman, G.E. Immunocytochemical evidence for anti-LHRH and anti-ACTH activity in the "F" antiserum. American Journal of Anatomy. 155, 139 -145, 1979.

Cross, B.A., and Dyer, R.G. Cyclic changes in neurons of the anterior hypothalamus during the rat etrus cycle and the effect of anestheia. In C.H. Sawyer and R.A. Gorski (Eds), Steroid Hormone and

Brain Function. UCLA Forum in Medical Sciences, No.P15, Los Angeles: University of California Press, pp 95-102, 1971.

Crowley, W.R., Jacobs, R., Volpe, J. Rodriguez-Sierra, Komisaruk, B. Analgesic effect of vaginal stimulation in rats: Modulation by graded stimulus intensity and hormones. Physiology and Behavior. 16, 483-488, 1976.

Del Rio, J., Naranjo, H.Y., Yang, T., and Costa, E. substance P-induced release of met-enkephalin from striatal and periaqueductal slices. Brain Research. 279, 121-126, 1983.

Domanski, E., Przekop, F., Skubiszewski, B. The role of the anterior regions of the medial basal hypothalamus in the control of ovulation and sexual behavior in sheep. Acta Neurobiologica Experimentia. 32, 753-762, 1972.

Dorner, G., Docke, F., and Moustafa, S. Differential localization of a male and female hypothalamic mating center. Journal of Reproductive Fertility. 17, 583-586, 1968.

Dorner, G., Docke, F., and Gotz, F., Male-like sexual behavior of female rats with unilateral lesions in the hypothalamic ventromedial nucleus region. Endokrinologie. 65, 133-137, 1975.

Dyer, R.G., Pritchett, C.J., and Cross, B.A. Unit

activity in the diencephalon of female rats durisng the estrous cycle. Journal of Endocrinology. 53, 151-160, 1972.

Dudley, C.A., Jamison, T.S., and Moss, R.L. Inhibition of lordosis behavior in the female rat by intraventricular infusions of prolactin and by chronic hyperprolactinemia. Endocrinology. 110, 677-679, 1982.

Edwards, D.A., and Mathews, D. The ventromedial nucleus of the hypothalamus and the hormonal arousal of sexual behaviors in the female rat. Physiology and Behavior.

Hagomen, W.D. and Brooks,D.C. Sexual behavior of female cats following lesions of the VMH. Anatomical Record 130: 414 (abstr.348) 1958.

Hall,M.E., and Stewart, J.M. Substance P and Antinociception. Peptides. 4, 31-35, 1983.

Hardy, D.F., and Debold, J.F. Effects of mounts without intromission upon the behavior of female rats during the onset of estrogen-induced heat, Physiology and Behavior. 7, 643-645, 1971.

Harlan, R.E., Shivers, B.D., Kow, L.M., Pfaff, D.W. Estrogenic maintenance of lordotic responsiveness: Requirements for hypothalamic action potentials. Brain Research. 268, 67-78, 1983a.

Harlan, R.E., Shivers, B.D., Kow, L.M., Pfaff, D.W.

Midbrain infusions of prolactin increase the estrogen dependent behavior, lordosis. Science 219, 1451-1453, 1983b.

Marlan, R.E., Shivers, B.D., Kow, L.M., and Pfaff, D.W. Intrahypothalamic colchicine infusions disrupt lordotic responsiveness in estrogen treated female rats. Brain Research. 238, 153-167, 1982.

Iversen, L.I., Jessell, T., and Kanazawa. Release and metabolism of substance P in rat hypothalamus. Nature. 201 81-83, 1979.

Jessell, T.M., and Iversen, L.L. Opiate analgesics inhibit substance P release from the rat trigeminal nucleus. Nature. 268, 549-551, 1977.

Komisaruk,B.R., Diakow, C. Lordosis intensity in rats in relation to the estrous cycle, ovariectomy, estrogen administration and mating behavior. Endocrinology. 93, 3, 548-557, 1973

Komisaruk, B.R., Ciofalo, V., and Latranyi, M.B. Stimulation of the vaginal cervix is more effective than morphine in suppressing a nociceptive response in rats. Advances in Pain Research and Therapy. Vol 1, 439-443, 1976.

Krieger, M.S., Conrad, L.C.A., and Pfaff, D.W. An autoradiographic study of the efferent connections of the VMN. Journal of Comparative Neurology. 183, 785-816, 1979.

Kow, L.M., Grill, H., and Pfaff, D.W. Elimination of lordosiss in decerebrate female rats: observations from acute and chronic preparations, Physiology and Behavior. 20, 171-174, 1978.

Kozlowski, G.P., Dees, W.L. Immunocytochemistry for LHRH neurons in the arcuate nucleus area of the rat: Fact or Artifact ? Brain Research 211, 233-245, 1984

Ljungdahl, A., Hokfelt, T., Nilsson, G. Distribution of subtance P -like immunoreactivity in the central nervous system of the rat: I Cell bodies and nerve terminals. Neuroscience 3 861-943, 1978.

Mc Clintock, M.K., and Adler, N.T. The role of the female during copulation in wild and domestic Norway rats (Rattus norvegicus). Behavior. 67, 67-96, 1978.

Mc Ewen, B.S., Davis, P.G., Parsons, B., Pfaff, D.W. The Brain as a Target for steroid hormone action In W.M. Cowan, Z.W. Hall, and E.R. kandel (eds) Annual Review of Neuroscience 2: 65-112, 1979

Malick, J.B., and Goldstein, J.M. Analgesic activity of substance P following intracerebral administration in rats. Life Sciences. 23, 835-844, 1978.

Malsbury, C.W., Kelly, D.B., and Pfaff, D.W. Responses of single units in the dorsal midbrain to

somatosensory stimulation in female rats. Proceedings of the Fourth International Congress of Endocrinology, Washington, June 18-24, 1972.

Malsbury, C.W., Kow, L.M., Pfaff, D.W. Effects of medial hypothalamic lesions on lordosis and other behaviors in female hamsters. Physiology and Behavior. 19, 223-237, 1977.

Malsbury, C.W., Daood, J.T. Sexual receptivity: critical importance of supraoptic connections of the ventromedial nucleus of the hypothalamus. Brain Research. 159, 451-457, 1978.

Malsbury, C.W., Miceli, M.O., and Scouten, C.W. Neural Basis of Reproductive Behavior. In The Hamster: Reproduction and Behavior. H.I. Siegel (Eds) New York, Plenum Press, in press.

Manogue, K., Kow, L-M, Pfaff, D.W. Lordosis in Female Rats Following Diencephalic and mesencephalic transections: role of the hypothalamus- midbrain fiber connections. Hormones and Behavior 21, 1980.

Modianos, D., Pfaff, D.W. Medullary reticular formation lesions and lordosis reflex in female rats. Brain Research. 171, 334-338, 1979.

Mohrland, J.S., and Gebhart, G.F. Substance P-induced analgesia in rat. Brain Research. 171, 556-559, 1979.

Morrell, J.I., and Pfaff, D.W. Characterization of estrogen-containing hypothalamic neurons by their axonal projections. Science 217, 1273-1275 1982.

Moss, R.L., and Dudley, C.A. Luteinizing Hormone Releasing Hormone (LHRH) : a role in extra-pituitary function. In: The Role of Peptides in Neuronal Function. Barker, J.L., Smith, T.G. (Eds). New York, Marcel Dekker Inc. pp 455-478, 1980.

Naranjo, J.R., Sanchez-Franco, F., and Del Rio, J. Blockage by met-enkephalin antiserum of analgesia induced by substance P in mice. Neuropharmacology. 21, 1295-1299, 1982.

Narahashi, T. Chemicals as tools in the study of excitable membranes. Physiology Reviews. 54, 813-889, 1974.

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

Pfaff, D.W. Interactions of steriod hormone with brain tissue: studies of uptake and physiological effects. In S. Segal et al. (Eds). The Regulation of Mammalian Reproduction. Springfield, III: Thompson, pp 5-22, 1973.

Pfaff, D.W. LHRF potentiates lordosis behavior in

hypophysectomized ovariectomized female rats. Science. 182, 1148-1149, 1979.

Pfaff, D.W., Lewis, L.C., Diakow, C., and Kreimer, M. Neural physiological analysis of mating behavior reponses as hormone sensitive reflexes. In E.Stellar, and J.M. Sprague (Eds). Progress in Physiological Psychology. 5 pp 253-297, 1972.

Pfaff,D.W., Montegomery,M., Lewis,C. Somaotsensory determinants of lordosis in female rats: behavioral definition of behavioral effect. Journal of Comparative and Physiological Psychology. 91, 134-145, 1977.

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

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

Pfaff, D.W., Estrogens and Brain Function: Neural analysis of a hormone-controlled Mammalian reproductive Behavior. Springer-Verlag New York, Hedelberg, Berlin, 1980.

Pfeifle, I.K., Shivers, M., and Edwards, D.A. Parasagittal hypothalamic knife cuts and sexual

receptivity in the female rat. Physiology and Behavior. 24, 145-150, 1980.

Quirion, R., Shults, C.W., Moody, T.W., Pert, C.B., Chase, T.N., O'Donohue, T.C. Autoradiographic distribution of Substance P receptors in the rat central nervous system. Nature. 303 714-716, 1983

Riskind, P., and Moss, R.L. Midbrain central gray: LHRH infusion enhances lordosis behavior in estrogen-primed ovariectomized rats. Brain Research Bulletin. 4 203-205, 1978.

Sakuma, Y., Pfaff, D.W. Facilitation of Female Reproductive Behavior From Mesencephalic Central Gray in the Rat. American Journal of Physiology. 237: R278-284, 1979a.

Sakuma, Y., Pfaff, D.W. Mesencephalic mechanisms for integration of female reproductive behavior in the rat. American Journal of Physiology. 237: R285-290, 1979b.

Sakuma, Y., and Pfaff, D.W. Modulation of the lordosis reflex of the female rats by LHRH, its antiserum, and analogs in the mesencephalic central gray. Neuroendocrinology. 36, 218-224, 1983.

Schally, A.V., Kastin, A.J., Arimura, A., Coy, E., Redding, K.L. Journal of Fertility. Suppl. 120, 119 1973.

Schwartz, N.B. A model for the regulation of

ovulation in the rat. Recent Progress in Hormone Research. 25, 1-55, 1969.

Silverman, A.J., Zimmerman, E.A. Pathways containing LHRH in the Mammalian brain. In: Brain Endocrine Interaction. III. Neural Hormones and Reproduction. 3rd Int. Symposium, Scott, D.E., Kozloyski, G.P. Weindle, A. (Eds). pp 83-96 1977.

Sirinathsinghi, D.J.S., Whittingon, P.E., Andsley, A., Fraser, H.M. B-endorphin regulates lordosis in the female rat by modulating LHRH release. Nature 301, 62-64, 1983.

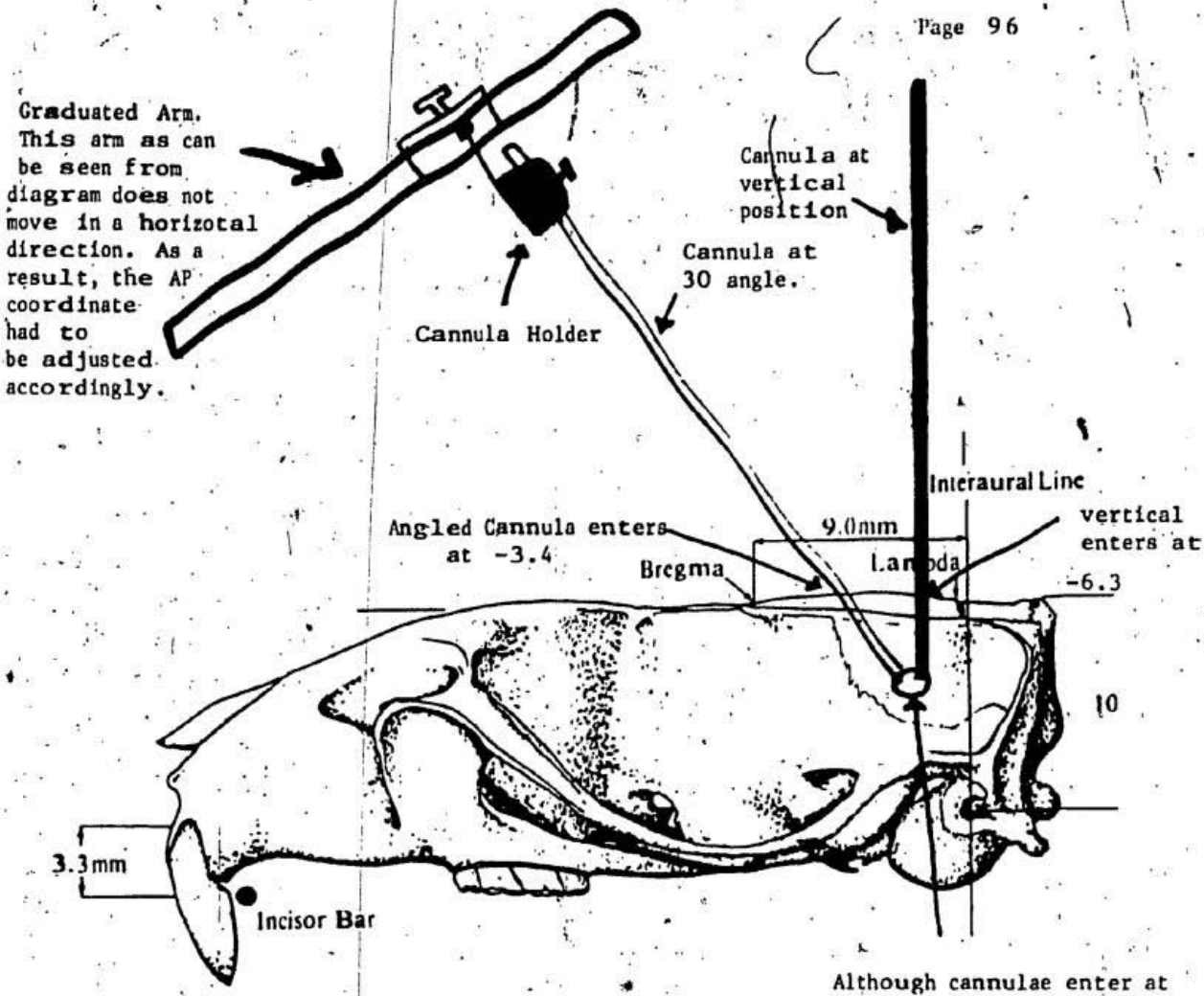
Tang, J., Chou, J., Yang, Y.T., and Costa., E Substance P stimulates the release of Met-enphalin from rat spinal cord. Neuropharmacolgy. 22, 1147-1150, 1983.

Whipple, B.,M.Ed, M, Komisaruk, B.R. Evidence that vaginal stimulation in women suppresses experimentally induced finger pain. Paper presented at the Conference of Reproductive Behavior. Pittsburgh June 84.

Yanase, M., and Gorski, R.A. Sites of estrogen and progesterone facilitation of a male and female hypothalamic mating center. Journal of Reproductive Fertility. 17, 583-586, 1976.

Young,W.C. The Hormones and Mating Behavior. In W.

Young (ed) Sex and Internal Secretions, Vol.2,
Baltimore, Williams and Wilkins, 1961.



Although cannulae enter at different areas, ultimately, they end up in the central gray at app. the same place.

Apendix A

Primarily this adjustment was done to enable the investigator to put dummy cannula on the guide cannula without touching the other.