THE CENTRAL ADMINISTRATION OF OXYTOCIN AND OXYTOCIN ANALOGS
TO STEROID-PRIMED FEMALE RATS: AN INVESTIGATION OF THE
EFFECTS OF BRAIN SITE, DOSE, RECEPTOR TYPE, AND TIME
PARAMETERS ON THE GENERATION OF LORDOSIS BEHAVIOR

by

H. GEORG SCHULZE
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Department of **Psychology**

The University of British Columbia
Vancouver, Canada

Date 12 August 1991
Abstract

Oxytocin plays an important role in the orchestration of many behaviors, including reproductive behaviors, in the female rat. Although it is known to influence sexual receptivity, relatively little is known about the central sites of action of oxytocin and how these contribute to the generation of lordosis. Furthermore, it is not clear what the mechanisms of action of this peptide at these sites may be, and how temporal factors influence oxytocinergic activity. The purpose of this thesis is to investigate the role of oxytocin in the generation of lordosis behavior in the female rat from a variety of perspectives in order to gain a broader understanding of this behavior.

The administration of low doses of oxytocin to the lateral ventricle of female rats treated with 5 μg estradiol benzoate and 150 μg progesterone inhibited lordosis behavior while higher doses had no effect. This contrasted with the administration of similar oxytocin doses to the medial preoptic area where low doses had little effect and higher doses tended to elevate primarily lordosis quotients. Higher oxytocin doses administered to the ventromedial hypothalamus extended lordosis durations but had a smaller effect on lordosis quotients. In the central nucleus of the amygdala, the same high doses also extended lordosis durations but not lordosis quotients.
These results imply that different brain sites may contribute to different aspects of the lordosis behavior: the medial preoptic area appears to be responsible for lordosis frequency (as measured by the lordosis quotient), the amygdala for lordosis duration, and the hypothalamus perhaps for lordosis initiation.

The infusion of an oxytocin agonist and antagonist into the lateral ventricles of steroid-primed female rats reduced lordosis quotients. The same agents infused into the medial preoptic area and the ventromedial hypothalamus did not produce any significant effects. These results are difficult to interpret but appear to be congruent with the notion that both the vasopressinergic system and the oxytocinergic system exercise control over lordosis behavior. The former system is inhibitory while the latter system appears to facilitate sexual behavior only when a certain threshold of neural activity has been reached. It furthermore appears likely that both peptides can act on the receptors of both systems.

When infused into the left lateral ventricles of steroid-treated female rats, the oxytocin agonist employed in these studies facilitated lordosis behavior 32 h after estradiol administration but not 48 h later when most reported investigations have been carried out. This implies that sufficient functional oxytocin receptors are induced within
32 h after estrogen treatment. It also raises the possibility that receptor induction and decay at different central sites may follow different time courses.

Taken together, these studies indicate that several brain sites contribute to the generation of lordosis and that both the vasopressinergic and oxytocinergic systems may be involved. Furthermore, these systems may be differentiated by temporal patterns of activation.
PREFACE

Some of the material contained in this thesis have been published, submitted for publication, or are being considered for submission.

In particular, the results of Experiments 2 and 4 have been published. The relevant reference is the following:

The results of Experiments 1 and 3 have been submitted:
Schulze, H. G.; Gorzalka, B. B. Low concentrations of oxytocin suppress lordosis when infused into the lateral ventricle of female rats. Endocrine Regulations: manuscript submitted.

The results from Experiments 7 and 8 are being considered for publication and may be submitted after completion of this thesis. The submitted work is expected to be a duplication of Section IV and will be reported by the same authors in the same order.

All the material published, submitted for publication and considered for publication is the work of the first author.
The second author was responsible for careful revisions of the material and for input regarding style, clarity, and presentation. In addition, all of the work was carried out in the laboratory of and with the funds available to the second author.

Signed: ..................

B.B.GORZALKA

Signed: ..................

H.G.SCHULZE
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INTRODUCTION

Oxytocin (OT), which in 1953 became the first peptide to be synthesized (du Vignaud et al. 1953), is known to be physiologically important in parturition and lactation and has also been shown to exert behavioral effects, namely in the expression of maternal behavior, learning and memory, drug tolerance and arousal (see reviews by Kovacs 1986, Argiolas and Gessa 1991). This peptide is synthesized peripherally in the ovaries (Ivell and Richter 1984, see also Ivell 1986), is secreted into the systemic circulation by the posterior pituitary (Hashimoto et al. 1988), is released in phase with progesterone (Flint and Sheldrick 1985; Schams et al. 1985) and its receptors are to be found in the smooth muscle of the female reproductive tract, in mammary myoepithelial cells (see Soloff 1985) and at some central sites (de Kloet et al. 1986). Centrally OT is synthesized mainly in the supraoptic and paraventricular nuclei (see Robinson 1983, Buijs et al. 1985). Moreover, there are oxytocinergic projections to the midbrain central grey (MCG), ventral tegmental area, dorsal vagal complex and spinal cord (Buijs et al. 1985). OT is transported to the spinal cord from the supraoptic and paraventricular nuclei (Camier et al. 1985) via fast axonal transport (White et al. 1986) which may imply a neurotransmitter role for this peptide in the spinal cord. In the spinal cord of newborn rats, iontophoretic application of OT induces a marked
depolarization of motorneurons (Suzue et al. 1981). OT is furthermore present in sensory neurons in the rat spinal cord (Kai-Kai et al. 1985). The presence of OT in motor and sensory systems may allow the peptide to play a role in the control of lordosis by influencing or modulating the behavior at spinal levels. In addition, OT levels in the portal blood (Sarkar and Gibbs 1984) and the posterior pituitary (Crowley et al. 1978) vary across the estrous cycle - levels being highest just before the onset of behavioral estrus. The importance of OT in reproductive functions and behaviors has kindled interest in this neuroactive substance as putatively being involved in sexual behavior.

Three components of sexual behavior in female mammals have been described: attractivity, proceptivity and receptivity (Beach 1976). A major aspect of receptivity in female rats is the lordosis reflex which consists of a concave arching of the back, elevation of the head and lateral deflexion of the tail, executed in response to a mount by a male rat. The lordosis quotient (LQ) is widely used as an index of receptivity and is expressed as a percentage of the number of lordoses shown in response to a set number (e.g. 10) of mounts (Hardy and DeBold 1972). Lordosis duration is another index of sexual receptivity and it is especially useful for measuring receptivity in hamsters (e.g. Lester and Gorzalka 1988). Although it has
been measured at least once in the female rat (see Madlafousek and Hlinak 1977), lordosis duration is seldom used as an index for receptivity in this animal and has not been used to investigate the effects of oxytocin on receptivity. Whether OT plays a role in the attractivity or proceptivity components of sexual behavior in female rats is not known, but it has been established that lordosis is facilitated by the administration of exogenous OT (e.g. Arletti and Bertolini 1985, Gorzalka and Lester 1987).

Recently, it has been demonstrated that OT is indeed involved in the expression of sexual behaviors in the rat (Arletti and Bertolini 1985, Caldwell et al. 1986, Gorzalka and Lester 1987, Hughes et al. 1987, Caldwell et al. 1989). However, at this stage relatively little is known about the central sites of action of this peptide and in particular about the mechanisms or modes of action at these sites. For instance, it is likely that the action of OT on sexual behavior, like the action on maternal behavior, is estrogen-dependent (see Fahrbach et al. 1985 for review). Several brain regions, particularly hypothalamic regions, have been shown to concentrate radiolabelled estradiol (Pfaff 1968, Pfaff and Keiner 1973). Estrogen also increases the number of OT receptors in the VMH when chronically administered (de Kloet et al. 1986, Johnson et al. 1989), stimulates the release of OT into the peripheral circulation (Yamaguchi et al. 1979), influences the electrical activity of OT neurons
(Negoro et al. 1973, Negoro et al. 1983), modifies the pattern of central OT innervation (Caldwell et al. 1988, Jirikowski et al. 1988) and may cause increased levels of OT mRNA (Miller et al. 1989). OT levels also vary in the paraventricular nucleus during the rat estrous cycle (Greer et al. 1986). Curiously enough, despite the involvement of OT in female reproductive functions, OT innervation of the brain does not appear to be sexually dimorphic as is that of arginine vasopressin (Buijs et al. 1985). The above evidence suggests a strong dependence of OT on the metabolic effects of estrogen. However, the precise nature of this dependence remains to be determined.

The second important ovarian hormone required for the full expression of sexual behavior in the female rat is progesterone (Boling and Blandau 1939, see Clemens and Weaver 1985 for a discussion of the role of gonadal hormones in sexual behavior). The actions of OT on lordosis are dependent on the presence of progesterone at some sites, e.g. the ventromedial hypothalamus (VMH) (Schumacher et al. 1989, Schumacher et al. 1990) and the lateral ventricle (Gorzalka and Lester 1987), but may not be at others, e.g. the medial preoptic area (Caldwell et al. 1989). In the VMH progesterone appears to facilitate lordosis by increasing the area of dispersion of OT receptors (Schumacher et al. 1989, Schumacher et al. 1990). Investigating the actions of progesterone on oxytocin effects on lordosis is complicated
by the synergistic effects of progesterone and estrogen on sexual behavior. For example, estrogen modulates progestin receptors in some brain areas but not in others (MacLusky and McEwen 1978) and can have progesterone-like effects on reproductive behavior (Parsons et al. 1984). Progesterone can also inhibit oxytocin release during vaginal distension (Roberts and Share 1970). At this stage, the role of progesterone in the expression of OT-mediated lordosis at other central sites is undetermined.

Ongoing investigations of the role of OT in sexual behavior focus on the central sites of action of OT (Caldwell et al. 1989) and the modes and mechanisms of action of OT at some of these sites (Caldwell et al. 1989, Schumacher et al. 1989, Caldwell et al. 1990, Schumacher et al. 1990). These issues also form the main objectives of the present set of experiments. In particular, the effects on lordosis of the administration of various concentrations of OT into a few selected estrogen-concentrating brain regions, known to induce OT receptors, will be investigated in animals treated with both the ovarian hormones estrogen and progesterone. In addition, the specific contribution of a particular site in the generation of the lordosis reflex will be further investigated through the administration of an OT agonist and antagonist. Finally, the results obtained will be contrasted to attempt a synthesis where the sites of action of OT, some of the modes and mechanisms of action of
OT at these sites and the contribution of these sites in generating the lordosis reflex will be related.

GENERAL METHODS

Animals and Surgery

Female Sprague-Dawley rats, from Charles River Canada, Inc., Montreal, were bilaterally ovariectomized through lumbar incisions while under sodium pentobarbital (Somnotol) anesthesia at about 70 days of age. Following surgeries, all animals were individually housed in standard laboratory wire mesh cages in a colony room maintained on a reversed 12h light/dark cycle and at 50% relative humidity, 21 C with free access to food and water.

Approximately one week after the ovariectomies, animals receiving central manipulations were anesthetized as before, placed in a stereotaxic instrument, and provided with a chronic intracranial cannula assembly using the stereotaxic atlas of Pellegrino et al. (1979) as guide. These procedures are described fully by Gray and Gorzalka (1979) and, except for placements in the lateral ventricle, were modified slightly, using a method outlined by Elliott (1986) to accommodate bilateral or double placements. Following surgeries, animals were returned to their home cages.
In the case of intraventricular cannulae, placements were tested about one week after surgery by infusion of 2 μg of angiotensin II (1 μg/μl) into the lateral ventricle of each animal. Only animals that showed a pronounced drinking response to this treatment were retained for experimentation. The accuracy of all other placements was verified by trial surgeries followed by the appropriate histologies. Once the histologies indicated correct placement of the cannulae, the remaining animals were subjected to surgery using the placement coordinates determined in the trial surgeries. In this manner about 20 animals per placement group were prepared.

Sexually vigorous Sprague-Dawley male rats served as studs. These were group housed, six to a cage, under conditions identical to those of the females.

Drug Procedures

Estradiol benzoate (EB) and progesterone (both from Sigma) were dissolved in warm peanut oil and administered subcutaneously (sc) in 0.1cc of vehicle. All OT infusions were made using an infusion pump (Sage instruments, model 341A) delivering at a rate of 4 μl/minute.
Behavioral Testing

Behavioral testing involved the presentation of an experimental female to a sexually vigorous male, previously habituated to the testing arena and given brief access to a fully receptive stimulus female, in a circular glass chamber measuring 29 cm in diameter, 45 cm in height and filled with about 4 cm of bedding material (Corncob Granules, Coeval Inc., St. Joseph, Ill.).

Testing was conducted during the middle period of the dark cycle in a testing room where a computer was used as an event recorder. Every experimental female was given 10 mounts with pelvic thrusting by a stud male. If a male did not mount after 2 minutes, the female was presented to another stud male until she had received 10 mounts or until 15 minutes had elapsed in which case the lordosis quotient was calculated on the number of mounts actually received by the female. Lordosis quotients were calculated as a percentage of the number of mounts that resulted in the lordosis posture. Lordosis postures were dichotomized and only postures with moderate or full dorsiflexion of the spine corresponding to the 2-point and 3-point lordosis scores of Hardy and DeBold (1972) were considered positive.
SECTION I

The administration of oxytocin to the cerebral ventricles.

Although central OT receptors have been identified (Brinton et al. 1984), there is a remarkable mismatch between sites of OT innervation and OT receptors (Buijs et al. 1985). This may indicate that release of OT into the cerebrospinal fluid (CSF) plays an important role in the transmission of oxytocinergic signals. OT is known to facilitate sexual receptivity in female rats when infused into the lateral cerebral ventricle (Arletti and Bertolini 1985, Caldwell et al. 1986, Gorzalka and Lester 1987, Caldwell et al. 1989). To date, no literature exists on the effects of OT infusions into the third ventricle. It is known that the infusion of behaviorally active substances into the lateral versus third ventricles can have differential effects. For example, Wilson and Hunter (1985) found that the infusion of serotonin into the lateral ventricle of female rats primed with low levels of estrogen and progesterone significantly facilitated lordosis but that the same dose infused into the third ventricle did not. Given the proximity of the third ventricle to the ventromedial hypothalamus (VMH) and the medial preoptic area (MPOA), sites which are known to be effective for the oxytocinergic facilitation of lordosis (Schulze and Gorzalka 1991; Caldwell et al. 1989; Schumacher et al. 1989), it seems reasonable to expect that this region would be more
sensitive than the lateral ventricle to infusion. Moreover, leakage of OT from infusion sites such as the VMH and MPOA into the nearby ventricle cannot always be ruled out. It remains to be determined whether infusions of OT into the third ventricle would have the same effect as infusions into the VMH or MPOA. Given a differential effect between infusion site employed and adjacent ventricle, one can be more confident about the resulting effect being due to the action of OT at the infusion site rather than due to leakage into the ventricle and subsequent action at a remote site. The present series of experiments were designed to examine the effects on lordosis of third and lateral ventricular OT administration.

Experiment 1

Experiment 1 was designed to compare the effect on lordosis of OT infused into the lateral ventricle with an identical dose infused into the third ventricle. The dose selected was relatively low, because higher doses would be less likely to reveal differentiation between the ventricles. With higher doses the probability and extent of diffusion or circulation of infused OT would be greater.
Materials and methods

Animals and surgery

Twenty female Sprague-Dawley rats were bilaterally ovariectomized and received intracranial cannulae aimed at the third and left lateral ventricles according to procedures described in the General Methods section. For the lateral ventricle the coordinates used for surgery were: 0.2 mm posterior, 1.8 mm lateral and 2.9 mm ventral; and for the third ventricle: 0.0 mm anterior, 0.0 mm medial and 9.0 mm ventral. Placement testing resulted in the exclusion of data from 11 animals from statistical analysis.

Drug procedures

Estradiol benzoate (5 µg) and progesterone (150 µg) were prepared and administered as described under General Methods. Estradiol benzoate was given 48 h and progesterone 4 - 6 h before behavioral testing. OT and angiotensin II (both from Sigma) were dissolved in physiological saline. Each animal received a total of 0.34 ng OT delivered in 4µl of vehicle or vehicle only administered 20-30 minutes before behavioral testing commenced.

The test animals were randomly assigned to one of four groups of five each. Each group received, in a
counterbalanced fashion, either OT or saline to either the left lateral ventricle or the third ventricle in such a way that each female received each treatment over the duration of the experiment. All groups were tested on the same day of the week at weekly intervals.

Behavioral testing

Behavioral testing was conducted as described under General Methods. Lordosis durations were not recorded.

Results

The results, illustrated in Figure 1, indicate an inhibition of LQ when OT is infused into the ventricles. A repeated measures analysis of variance revealed significant effects \( F(3,32) = 4.69, \) Huynh-Feldt \( p < 0.05 \). Tukey's post-hoc comparisons revealed a significant inhibition of lordosis in response to OT as compared to vehicle infusion into the lateral ventricles \( (p < 0.05) \). No statistically significant effect on LQ resulted from an OT infusion into the third ventricle.

Discussion

An OT dose of 0.34 ng administered to the left lateral ventricle significantly reduced the lordosis quotient of
Figure 1. The mean lordosis quotients ± S.E.M. for animals infused with 0.34 ng of oxytocin and saline vehicle into the lateral and third ventricle are shown. All animals were primed with 5 μg estradiol benzoate and 150 μg progesterone and received intracerebroventricular infusions 20-30 minutes before behavioral testing.
FIGURE 1.

VENTRICULAR INFUSION SITE

LORDOSIS QUOTIENT

Saline

Oxytocin
female rats treated with estradiol benzoate and progesterone. Administration of the same dose to the third ventricle had no effect. The inhibition was totally unexpected in light of previous research from this and other laboratories indicating that OT infusion into the lateral ventricle facilitated lordosis performance in female rats (Arletti and Bertolini 1985, Caldwell et al. 1986, Gorzalka and Lester 1987, Caldwell et al. 1989). In order to demonstrate that this inhibition was not simply a function of the specific doses of estrogen and progesterone, the present results were confirmed in 6 animals primed with lower doses of steroids (2 μg estradiol benzoate and 100 μg progesterone). The inhibitory effect was still present: LQ means ± S.E.M. were 28% ± 6.6 for the 0.34 ng OT infusion and 60% ± 17.0 for the saline control group; dependent t-test: t = 2.795, df = 5, p < 0.035. In order to further examine this surprising finding, we designed Experiment 3 to investigate the effects on lordosis behavior of various doses of OT, when infused into the left lateral ventricle of female rats.

Experiment 2

In previous studies of the effects of oxytocin on sexual receptivity, lordosis frequency was used as a dependent measure (e.g. Arletti and Bertolini 1985, Gorzalka and Lester 1987, Caldwell et al. 1989, Schumacher et al. 1989).
To date, the duration of the lordosis posture has not been employed, nor proposed, as a measure in such studies. Duration may provide a more sensitive measure of receptivity than the more frequently used LQ or may provide information about sexual behavior in the female rat not attainable through the use of the LQ score. In order to validate the use of lordosis duration (LD), we designed Experiment 2 to investigate the effect of different doses of progesterone on LD and to contrast that with LQ for the same animals.

Materials and methods

Animals and surgery

Forty female Sprague-Dawley rats were bilaterally ovariectomized as described in the General Methods section.

Drug procedures

Estradiol benzoate and progesterone were dissolved in warm peanut oil and administered subcutaneously in 0.1 cc of vehicle. The experimental animals were then randomly assigned to four groups of ten animals each. All groups were tested on the same day. All animals received 10 μg estradiol benzoate 48 h before behavioral testing and either oil vehicle, 100 μg, 300 μg or 500 μg progesterone 4-6 h before testing.
Behavioral testing

Behavioral testing was conducted as described in the General Methods section.

In addition to lordosis frequency, the duration of every lordosis was also recorded and a LD calculated for each animal corresponding to the average duration. LD was considered to be 0 when a female rat did not show any lordoses.

Results and discussion

Statistically significant correlations were obtained between progesterone dose and LQ (r = 0.687, Bonferroni adjusted p < 0.001), progesterone dose and LD (r = 0.486, Bonferroni p < 0.01) as well as LQ and LD (r = 0.813, Bonferroni p < 0.001). Figure 2 displays the LQ and LD values for the four groups tested.

The high correlation between LQ and LD suggests that LD may be as valid a measure of sexual receptivity in the female rat as LQ. However, it appears to be somewhat less sensitive than LQ to the effects of progesterone, suggesting that it is not simply a function of the LQ measure. The possibility exists that for some pharmacological treatments,
Figure 2. Mean lordosis fraction (LQ/100) ± S.E.M. and mean lordosis duration ± S.E.M. as a function of progesterone dose administered subcutaneously 4-6 h before testing to female rats primed with 10 μg estradiol benzoate 48 h before testing.
BEHAVIORAL SCORE

DOSE OF PROGESTERONE (μg)

FIGURE 2.
LD could be a more sensitive measure than LQ. We therefore decided to generally assess both measures of female sexual receptivity.

Experiment 3

Experiment 3 was conducted as a dose-response study to investigate the effects on lordosis of various doses of OT when infused into the left lateral ventricle.

Materials and methods

Animals and surgery

Forty animals were ovariectomized and cannulated as described for Experiment 1. Instead of receiving two cannulae, each animal received a single cannula placed in the left lateral ventricle.

Drug procedures

Each animal was primed with 5 μg estradiol benzoate and 150 μg progesterone as described for Experiment 1 and received an infusion of one of the following - saline vehicle, 0.34, 3.4 or 34 ng of OT. Infusion volumes and rates were as described in Experiment 1. Before every testing session, animals were randomly assigned to one of
the four drug treatment groups, in a manner which resulted in approximately equal groups.

Behavioral testing

Behavioral testing was conducted blind at weekly intervals according to procedures described in Experiment 1. A microcomputer with custom written software was employed as an event recorder and both the lordosis frequency and the duration of each individual lordosis response were recorded. LQ and LD scores were calculated as before. Based on procedures used to confirm ventricle placements described in Experiment 1, the data from some animals were eliminated. This resulted in group sizes of 10, 11, 11, and 11 for the 0 - 34 ng OT doses, respectively.

Results

Inspection of the data presented in Figure 3 suggests that OT, when infused into the lateral ventricle, had an inhibitory effect on LQ. An analysis of variance confirmed that there was a significant treatment effect \( F(3,39) = 3.96, p < 0.05 \). A significant inhibition (Dunnett, \( p < 0.05 \)) of LQ occurred in response to 0.34 ng OT but not in response to 3.4 or 34 ng OT. None of the doses tested had a statistically significant effect on LD (means ± S.E.M. were
Figure 3. Dose-response curve of the effects on lordosis of oxytocin infusions into the left lateral ventricle of female rats primed with 5 µg estradiol benzoate and 150 µg progesterone is illustrated. Infusions were performed 20-30 minutes before behavioral testing. Shown are the mean lordosis quotients ± S.E.M.
1.28 ± 0.08, 1.12 ± 0.14, 1.19 ± 0.16 and 1.21 ± 0.11 for the 0 - 34 ng doses, respectively).

Discussion

An OT dose of 0.34 ng infused into the lateral ventricle significantly inhibited the frequency of lordosis. This confirmed the results obtained in Experiment 1. There was, however, no indication of a facilitation. This may have been due to the relatively low OT doses used in Experiment 3 (0.34, 3.4 and 34 ng) as opposed to those employed by others (e.g. 100 and 500 ng) (Caldwell et al. 1989). Although 500 ng OT has been shown to facilitate lordosis when infused into the lateral ventricle, 100 ng OT produced a non-significant facilitation in the same study (Caldwell et al. 1989). In a further attempt to demonstrate a facilitatory OT effect, we infused a higher dose of OT (340 ng) into the lateral ventricles of 9 female rats primed with 3 µg estradiol-benzoate and 150 µg progesterone. This resulted in a LQ of 38% ± 13.0 as compared to a score for the saline treatment of 42% ± 10.4. These differences were not statistically significant.

General discussion

The infusion of a relatively low dose of OT (0.34 ng) into the left lateral ventricle of female rats significantly
attenuated the frequency of lordosis. The same dose when applied to the third ventricle was ineffective. Of all the OT doses (0.34 - 340 ng) employed, none facilitated lordosis when administered to the lateral ventricle of estrogen and progesterone treated animals.

There are several reasons why OT infusions into the lateral and the third ventricle might be expected to differ in their effects on lordosis. Although the ventricular system of the rat is interconnected and cerebrospinal fluid is actively circulated through the system, the proximities of the different ventricles to specific brain structures vary. In particular, the third ventricle is much closer than the lateral ventricles to the hypothalamus and the medial preoptic area. The third ventricle is bordered by the paraventricular and supraoptic nuclei which contain OT (Armstrong 1985). Dendrites of paraventricular neurons pass along this ventricle in the mouse (Castel and Morris 1988) and immunoreactive vasopressin and OT axons project into the third ventricle under some conditions of salt loading (Dellmann et al. 1988). The above considerations would also lead one to expect infusions of OT into the third ventricle to be more likely to produce an effect on lordosis than a similar treatment of the lateral ventricle. However, the results of Experiment 1 proved contrary to this expectation. On the other hand, differential ventricular effects of OT on lordosis are consistent with the literature on serotonin and
ß-endorphin. Infusion of 10 µg serotonin into the lateral, but not the third, ventricle of female rats facilitated lordosis, whereas 100 µg serotonin was ineffective in either ventricle (Wilson and Hunter 1985). A dose of 2 µg ß-endorphin inhibited lordosis when administered into the third (Wiesner and Moss 1986) but not the lateral ventricle (Pfaus and Gorzalka 1987). It may be that when infused into the lateral ventricle, OT more readily gains access to undetermined central sites associated with the inhibition of lordosis. Because known central sites of action of OT on lordosis are facilitatory (Caldwell et al. 1989, Schumacher et al. 1989, Schulze and Gorzalka 1991), this appears to be the first evidence suggestive of an inhibitory site.

It is surprising that OT infusions into the lateral ventricle should produce a facilitation of lordosis (Arletti and Bertolini 1985, Caldwell et al. 1986, Gorzalka and Lester 1987, Caldwell et al. 1989) as well as an inhibition (Experiments 1 and 2) depending on the OT dose administered. This finding is reminiscent of the inhibitory effect on lordosis of a lower dose of ß-endorphin and the facilitatory effect of a higher dose (Pfaus and Gorzalka 1987). There are four possibilities that we wish to address concerning the opposite effects of OT on female sexual receptivity: (i) that an inhibitory site exists; (ii) that an OT autoreceptor exists; (iii) that OT at some concentrations may modulate other peptidergic systems affecting lordosis and (iv) that
the effects of OT on lordosis may be dependent on the progesterone and estrogen dose.

As pointed out above, known central sites of action of OT on lordosis are facilitatory (Caldwell et al. 1989, Schumacher et al. 1989, Schulze and Gorzalka 1991) and the inhibition reported here suggests that an inhibitory site exists. At relatively low doses of OT, the inhibitory site may be activated, while at higher doses OT diffuses to facilitatory sites, thus masking the inhibition. It seems likely that this putative inhibitory site is not in the immediate vicinity of the third ventricle.

The existence of an OT autoreceptor might explain both the inhibition and the facilitation seen in response to infusions into the lateral ventricles. Suppose that OT functions to facilitate sexual receptivity in female rats. At low concentrations of OT the autoreceptor may be activated, inhibiting the facilitatory effects of OT on lordosis. It appears that activation of V$_2$ receptors on oxytocinergic cells down-regulates OT secretion (Cheng and North 1989). Furthermore, OT may bind to these V$_2$ receptors (Hruby and Smith 1987). If high levels of activity of OT neurons are necessary for the expression of lordosis, this inhibition of OT secretion will then inhibit lordosis. At higher doses of OT such activation does not occur and no inhibition would be seen. This is supported by evidence
indicating that high levels of activity of OT cells, or the infusion of OT into the third ventricle, cause the recruitment of other cells (Belin and Moos 1986) possibly through the local release of OT (Mason et al. 1986). Therefore, high doses of OT may trigger such recruitment resulting in a facilitation of lordosis. Thus vasopressin V2 receptors located on OT neurons may function as OT autoreceptors. Alternatively, it is quite possible that OT normally inhibits mating in the female rat and that lower (and more physiological) doses of OT when applied centrally reflect this. At higher doses of OT, the autoreceptors would become activated and shut the inhibitory system down, resulting in a facilitation of sexual receptivity.

The activation of other peptidergic systems provides another explanation for the dual effect of OT. Although at this stage only a single central OT receptor-type is known to exist, OT also binds to vasopressin V3 receptors (Antoni 1987) and other vasopressin receptors (Hruby and Smith 1987). This raises the possibility that OT may bind to yet other types of receptors. The different effects of OT on lordosis may be mediated through activation of different types of receptors in a manner analogous to the inhibitory and excitatory serotonergic receptor subtypes regulating male and female sexual behavior (Gorzalka et al. 1990). It is conceivable, for example, that the prolyl-leucyl-glycinamide fragment of the oxytocin molecule activates a
facilitatory site (Gorzalka et al. 1991) whereas another fragment of the oxytocin molecule might, depending on the brain site and steroid milieu, activate an inhibitory receptor. Moreover, at certain concentrations, OT may be acting on the receptors of other peptides to involve these peptidergic systems in the expression of lordosis. Recent work by Caldwell et al. (1990) involving the vasopressin antagonist [d(CH2)5 Tyr(Me)]-AVP indicates that blocking activity at vasopressin V1 receptors may facilitate lordosis through the lifting of a tonic inhibition. However, the same antagonist used by these authors has been shown to act as a partial V3 agonist (Antoni 1987). Therefore it remains possible that the effects of this antagonist on lordosis behavior are due to its action at V3 receptors. Pituitary V3 receptors mediate the release of adrenocorticotrophic hormone which, when peripherally administered, increases lordosis (deCatanzaro et al. 1981). The facilitation seen with OT in some studies may partially reflect the effects of adrenocORTical activation.

The effects of OT on other peptidergic systems implicated in lordosis behavior may involve, in addition to the activation of different receptors, other mechanisms as well. For instance, OT is considered to be a stress hormone and is released in response to various stressors: hyperosmolality (Forsling and Brimble 1985), immersion in water (Lang et al. 1983), and immobilization (Lang et al. 1983, Gibbs 1984). In
agreement with this view, it has been shown that OT and vasopressin function as corticotropic releasing factors (CRF) (Antoni 1987), resulting in increased ACTH levels. Moreover, in some brain areas, CRF and OT are colocalized (see Sawchenko and Swanson 1985 for discussion) and one may expect them to be released concomitantly. Both CRF (Sirinathsinghi 1985) and ACTH (deCatanzaro et al. 1981), however, centrally inhibit lordosis in female rats. In addition, OT releases prolactin centrally (Arey and Freeman 1989) and prolactin in turn releases OT (Sarkar 1989). Chronic hyperprolactinemia and the intraventricular infusion of prolactin inhibit lordosis behavior in female rats (Dudley et al. 1982). This raises the possibility that OT facilitates the direct activation of functional oxytocinergic brain sites (e.g. Caldwell et al. 1989, Schumacher et al. 1989, Schulze and Gorzalka 1991), but may inhibit lordosis behavior indirectly through the activation of other peptidergic systems. The indirect effects of OT may be manifest only at relatively low concentrations since high concentrations, resulting in the activation of facilitatory oxytocinergic systems, may override them.

Other possible factors contributing to an inhibitory effect of oxytocin infusions into the lateral ventricle are the dose and duration of estrogen treatment and the presence or absence of progesterone. In priming regimens where an acute dose of estrogen was followed by an acute dose of
progesterone, OT facilitated lordosis when the EB dose was 10 μg (Arletti and Bertolini 1985, Gorzalka and Lester 1987) but inhibited lordosis when the EB dose was 2-5 μg (Experiments 1 and 3). Moreover, in a priming regimen where progesterone was absent and even lower doses of EB were administered chronically for three days, OT was facilitatory (Caldwell et al. 1986, Caldwell et al. 1989). Therefore, the observation of both a facilitation and an inhibition in animals primed with identical steroid doses may be unlikely. This could explain our failure to see a facilitation of lordosis with 340 ng OT infused into the lateral ventricles of female rats. Now that an inhibitory effect of a low dose of OT has been demonstrated, further investigations are needed to reveal the possible interactions between OT dose, estrogen dose and progesterone.

SECTION II

The administration of oxytocin to brain regions that show increases in the number of oxytocin receptors in response to estrogen treatment.

Schumacher et al. 1990), mesencephalic central grey (MCG) (Sakuma and Pfaff 1979a, Sakuma and Pfaff 1979b, Arendash and Gorski 1983, Harlan et al. 1983, Rothfeld et al. 1986) and amygdala (Lisk and Barfield 1975) are all sites that have been shown to concentrate radiolabelled estradiol and to exert control over lordosis, the major index of sexual receptivity.

Some of the effects of estrogen on lordosis may involve interaction with OT. For instance, estradiol influences OT immunoreactivity in the MPOA (Caldwell et al. 1988), OT immunoreactive levels in the MPOA of rats treated with estradiol and progesterone are increased as a result of mounting and the number of cell bodies stained for OT are decreased in the same animals (Caldwell et al. 1989) - perhaps indicating increased OT release. Also, OT when administered to this nucleus facilitates lordosis in female rats treated chronically with estrogen (Caldwell et al. 1989). In the VMH estradiol increases the number of OT receptors when chronically administered (de Kloet et al. 1986, Johnson et al. 1989) and increases levels of OT mRNA (Miller et al. 1989). Estrogen treatment also modifies the pattern of central OT innervation (Rhodes et al. 1981, Jirikowski et al. 1988, Caldwell et al. 1988) and furthermore, OT levels vary in the paraventricular nucleus as a function of the estrous cycle (Greer et al. 1986). The MCG is an area that may be affected by changes in the OT
innervation in response to the presence of estrogen. It is known to receive projections from the VMH (see Andrezik and Beitz 1985) and OT fibers from the paraventricular nucleus (Buijs et al. 1985).

Although progesterone interacts synergistically with estrogen for the full expression of sexual behavior (Boling and Blandau 1939), some controversy exists as to whether the OT facilitation of lordosis behavior is progesterone dependent. Supporting evidence for dependence indicates that progesterone administration increases levels of immunoreactive OT in the MPOA and in fibers lateral to the VMH and decreases them elsewhere (Caldwell et al. 1986, Caldwell et al. 1988). In agreement with this evidence, dependence has been demonstrated in several studies of sexual receptivity (Arletti and Bertolini 1985, Gorzalka and Lester 1987). Such studies suggest that the mechanism of action of OT likely involves more than mimicking the effects of progesterone.

In a recent study Caldwell et al. (1989) found that OT infusions into the MPOA and lateral ventricles (LV), but not into the VMH, MCG, and ventral tegmental area (VTA), facilitated lordosis in female rats treated chronically with estradiol benzoate. However, in another study published at the same time, it was found that progesterone increased the dispersion of OT receptors in the VMH and that OT infusions
into the VMH enhanced the lordosis quality, but appeared to have less of an effect on the lordosis quotient (LQ), a measure of lordosis frequency, in rats treated with estradiol benzoate and progesterone (Schumacher et al. 1989). Since natural estrus involves both of the ovarian steroids, and Caldwell et al’s (1989) investigation of OT administration to the MPOA, VMH, LV, VTA and MCG employed rats not treated with progesterone, we investigated the effects of OT infusions into the MPOA, VMH and MCG in animals treated with both estradiol benzoate and progesterone. This rationale is all the more compelling given that progesterone can strongly influence the effects of other behaviorally active substances. For example, some selective serotonin receptor agonists are facilitatory in the presence of estrogen and inhibitory in the presence of both estrogen and progesterone (Gorzalka et al. 1990). In the studies by Caldwell et al. (1989) and Schumacher et al. (1989), relatively high doses of OT were infused centrally. In light of the preceding discussion, we expected progesterone to have a permissive or facilitative action on the effect of central OT infusions on lordosis and consequently examined the effects of lower OT doses covering four orders of magnitude. Finally, the two cited studies (Caldwell et al. 1989, Schumacher et al. 1989), taken together, suggest that oxytocinergic stimulation of the MPOA may have primarily an effect on lordosis frequency while the VMH may be integral to other aspects of the lordosis
posture. In order to further investigate this possibility, we measured both lordosis duration (LD) and lordosis frequency in the present study.

Experiment 4

This study was designed to measure the effects on the LQ and LD of various doses of OT administered to the MPOA, VMH and MCG of female rats primed with estradiol benzoate and progesterone.

Materials and methods

Animals and surgery

Female Sprague-Dawley rats were ovariectomized and cannulated as described under General Methods. Following surgeries, animals were returned to their home cages. As described above, female rats were ovariectomized and received cannulae bilaterally aimed at the medial preoptic area (n = 20), ventromedial hypothalamus (n = 20) and mesencephalic central grey (n = 20). Each group was then randomly subdivided into five groups of four animals each. All subgroups with implants in the same area were tested on the same day at weekly intervals using a counterbalanced design where one of four different concentrations of OT or saline vehicle was infused intracranially. The accuracy of
all placements was verified through the use of histological procedures and only the data from animals with correct cannula placements were retained for statistical analyses.

Drug procedures

All experimental animals were given 3 μg of EB and 150 μg of progesterone dissolved in peanut oil vehicle as described earlier. OT (Sigma) was dissolved in physiological saline in four different concentrations, 0.085, 0.85, 8.5 and 85 ng/μl. All OT infusions were administered as described under General Methods. The MPOA, VMH and MCG implanted animals were bilaterally infused with a total of 1 μl of solution per side per testing session. Infusions were performed 30 minutes before testing started.

Behavioral testing

Lordosis behavior was recorded as described in Experiment 2 and the LQ and LD accordingly calculated for the MPOA and VMH. Lordosis frequency scores for the MCG were taken manually and the LQ was calculated as described in Experiment 2. All testing was conducted blind.
Results

MPOA

Relative to saline, an OT dose of 8.5 ng significantly (p < 0.01) facilitated lordosis behavior as expressed by LQ scores when infused into the MPOA (Wilcoxon signed ranks test, z = 2.923). The other OT doses did not produce a significant effect when compared to the control condition.

The same OT dose of 8.5 ng infused into the MPOA of EB and progesterone primed female rats significantly (p < 0.05) extended the duration of lordosis (Wilcoxon signed ranks test, z = -2.261) compared to the saline control values. No other OT dose produced a significant effect when compared to the control condition.

VMH

None of the OT doses caused a significant increase in LQ scores vis-a-vis the control infusions using the Wilcoxon signed ranks test. However, all durations were significantly extended: the 0.085 ng (z = 2.380, p < 0.05), the 0.85 ng (z = 1.960, p < 0.05), the 8.5 ng (z = 2.033, p < 0.05) and the 85 ng (z = 2.240, p < 0.05) OT doses produced increases in lordosis duration compared to the control infusions.
MCG

None of the OT doses infused into the MCG proved to have a significant effect on LQs.

These results are represented in Figure 4.

VMH versus MPOA

It is evident from the preceding results that the effect of OT infusions into the VMH on LD is more dramatic than when infused into the MPOA. When combining all the duration scores of all the OT treatments for the VMH and the MPOA, the mean lordosis duration + S.D. scores for the two sites were 0.928 ± 0.238 and 0.752 ± 0.356 respectively. A Mann-Whitney U test indicated that the infusion of OT into the VMH was significantly (p < 0.05) more effective in increasing LDs than OT infusions into the MPOA. In contrast, there were no significant differences in LQ values for the VMH (all doses) and the MPOA (all doses).

General discussion

Infusions of oxytocin into the VMH and MPOA were effective in increasing the lordosis quotient and the lordosis duration of female rats treated with both estradiol benzoate and progesterone. None of the oxytocin doses used
Figure 4. The top two panels show the mean lordosis fraction (lordosis quotient/100) ± S.E.M. and mean lordosis duration ± S.E.M. as a function of oxytocin dose infused in 1 µl of vehicle into the medial preoptic area (MPOA - top panel) and the ventromedial hypothalamus (VMH - middle panel) of female rats primed 48 h before testing with 3 µg estradiol benzoate and 4-6 h before testing with 150 µg progesterone. The bottom panel shows the mean lordosis fraction ± S.E.M. following oxytocin infusion into the mesencephalic central grey (MCG).
had an effect on LQ when infused into the MCG. Furthermore, the VMH was more sensitive to the effects of oxytocin on LD than the MPOA. These results were obtained using lower OT doses (0.085 - 85 ng) than those used by Caldwell et al. (1989) (100 and 500 ng) and Schumacher et al. (1989) (100 ng).

Consistent with the findings of Caldwell et al. (1989), we observed that OT infusion into the MPOA facilitated sexual receptivity in the female rat and that a lower dose of OT was more effective than the highest OT dose used. Our findings reveal a facilitation at a rather lower OT dose (8.5 ng) than their lowest dose (100 ng). In view of the fact that in our experiment the 85 ng OT dose failed to produce an effect while their 100 ng dose did, it appears that one of the actions of progesterone may be to shift the dose response curve toward the lower concentrations. Perhaps progesterone increases the sensitivity of the MPOA oxytocin system permitting a behavioral effect of lower doses of OT.

We also found an effect of OT when administered to the VMH in EB and progesterone primed female rats, a result which differs with that of Caldwell et al. (1989) - perhaps due to the fact that their animals received no progesterone. Examination of Figure 4 suggests that at the higher doses, OT facilitated lordosis when infused into the VMH. Because the 8.5 and 85 ng groups did not differ significantly from
each other (paired samples t-test, $t = 0.084$, df = 7, $p = 0.935$) these two groups were combined. Their combined mean was significantly elevated with respect to the control infusion (one-tailed independent t-test: pooled variances $t = -2.011$, df = 22, $p < 0.05$; separate variances $t = -1.858$, df = 11.6, $p < 0.05$) indicating a facilitatory effect of OT in the VMH on the LQ.

Examination of Figure 4 further reveals that at the higher doses OT seemed to establish a plateau by elevating LQs by about 27%. It is interesting to speculate why this should happen at submaximal levels. One possibility is that the spread of OT receptors induced by progesterone in this brain area (Schumacher et al. 1989) is proportional to the amount of progesterone administered. If one further postulates that the LQ exhibited by a particular animal is also proportional to the spread of OT receptors induced (which could make more OT receptors accessible to molecules), a possible mechanism to account for this effect can be established bearing in mind that a submaximal dose of progesterone (150 $\mu$g/animal) was given. It is also possible that the small EB dose (3 $\mu$g/animal) lead to the synthesis of a submaximal number of OT receptors and that they were fully spread when given the current progesterone dose. The ceiling effect thus may reflect the smaller number of accessible or available OT receptors. It would therefore
seem worthwhile to examine the effects of intracranial OT at a variety of estrogen and progesterone doses.

In the MCG none of the OT doses tested had an effect on the LQ. This is consistent with the findings in animals treated with estrogen only (Caldwell et al. 1989). It seems likely that the role of the MCG in lordosis is independent of an OT mechanism.

OT, when infused into the VMH and MPOA, increased the duration of lordosis relative to vehicle infusions. Of these two sites, the VMH appeared to be the most sensitive to the effects of OT infusion on lordosis duration. If the LD is in some way related to lordosis quality, then an increase in LD due to infusions into the VMH supports the finding of Schumacher et al. (1989). Schumacher et al. (1989) found a significant effect of OT on lordosis quality but did not report a significant increase in LQ following infusion into the VMH. Furthermore, OT infusions at the single most effective dose precipitated a much more robust effect on LQ scores in the MPOA (p < 0.01) than in the VMH (p < 0.1). This supports the position of Caldwell et al. (1989) that the MPOA is the most sensitive brain area for the facilitatory effects of OT on LQ. We suggest, in the light of the findings of Schumacher et al. (1989), Caldwell et al. (1989) and those presented here, that the MPOA and VMH differentially control aspects of the lordosis posture -
more specifically - that the MPOA may predominantly control the frequency of lordosis expression while the VMH controls lordosis quality and duration.

An examination of the data in Figure 4 suggests that activity in the MPOA associated with increased lordosis frequency is more likely to be phasic, that is, to occur when a specific 'state' of OT neurotransmission (here reflected by the OT dose) has been reached. In contrast, it appears that the VMH may provide a more tonic signal, that is, once this nucleus is activated to cause an increase in lordosis duration, it tends to remain activated.

The hierarchical relationships between these two nuclei in exerting control over lordosis are not clear at this stage. Given the fact that LD is not merely linearly related to LQ, there are at least three basic possible relationships between the MPOA and the VMH. These are: (i) that both independently provide signals to a third site, e.g. the MCG, where the incoming signals are integrated; (ii) that a signal originates in the MPOA, is passed on to the VMH where duration information is incorporated into the signal before being relayed further and (iii) that a signal originates in the VMH containing duration information, is passed along to the MPOA which gates or superimposes frequency information on the signal before being relayed downstream. These relationships remain to be tested.
It appears that OT when applied to the MPOA increases LQ scores in estrogen-treated rats whether progesterone is present (Experiment 4) or not (Caldwell et al. 1989). By contrast, it appears that the effects of OT in the VMH vary with progesterone treatment. In the absence of progesterone, OT fails to increase LQ (Caldwell et al. 1989, Schumacher et al. 1989) or lordosis quality (Schumacher et al. 1989). In the presence of progesterone, OT infused into the VMH increases LQ (Experiment 4), LD (Experiment 4) and lordosis quality scores (Schumacher et al. 1989).

Although Caldwell et al. (1989) observed a facilitation of lordosis in the absence of exogenous progesterone following infusion of OT into the MPOA and lateral ventricles, a significant effect was first observed on the fourth repeated test, the fourth test occurring approximately 90 minutes after the first. It should be noted that repeated tests occurring at short intervals are likely to increase the release of adrenal steroids, including progesterone. In ovariectomized, estrogen-treated animals, progesterone originating in the adrenals may have behavioral consequences (Whalen et al. 1975). Furthermore, repeated testing causes an increase in receptivity which appears to be adrenally mediated (Larsson et al. 1974). Consistent with this interpretation are the findings of Gorzalka and Lester (1987) indicating that in the absence of repeated tests, OT
infused into the lateral ventricles of estrogen-primed rats was ineffective in facilitating sexual receptivity, except when progesterone was present. It would therefore seem reasonable, in studies employing a repeated test paradigm, to consider a possible role for adrenal steroids.

The amygdala is also an estrogen-concentrating region (Pfaff and Keiner 1973), sexually dimorphic (Meany et al. 1985), responds differently to testosterone propionate in male and female neonatal rats (Takeshita et al. 1987), shows estrous cycle variations in peptide content (Frankfurt et al. 1986) and has been shown to affect lordosis (Lisk and Barfield 1975, McGinnis et al. 1985).

In response to estrogen administration, a two-fold increase in OT receptors are seen in the central nucleus of the amygdala (ACE) (de Kloet et al. 1986). Furthermore, OT fibers project to the amygdala (Buijs et al. 1985). It is possible then that the amygdala contributes to lordosis behavior via activation of the oxytocinergic system. The effects on lordosis of various doses of OT infused into the ACE of estrogen and progesterone primed rats were investigated in Experiment 5.
Experiment 5

The effects on lordosis behavior of the administration of various doses of OT into the central nucleus of the amygdala in female rats primed with estrogen and progesterone.

Materials and methods

Another set of 20 Sprague-Dawley female rats were used in Experiment 5. All surgical and drug procedures were exactly as described for Experiment 4, except that cannulae were aimed at the central nucleus of the amygdala and that females were primed with 10 μg of estradiol benzoate and 100 μg progesterone. In all other respects, animals were assigned to groups, tested and the placements verified in a manner identical to that described for experiment 4. Histological procedures resulted in the retention of data from 7 animals for statistical analysis.

Results and discussion

None of the OT doses infused into the ACE had a significant effect on LQ scores when compared with the control infusions using the Wilcoxon signed ranks test. However, the infusion of 8.5 ng OT did significantly extend lordosis duration when compared with the control infusion (z = 2.113, p < 0.05). A significant correlation was obtained
between OT dose and LD (Pearson r = .343, df = 33, p < 0.05) but not between OT dose and LQ (Pearson r = .071, df = 33). These results are shown in Figure 5.

The results from Experiment 5 strongly suggest that LQ and LD are independently regulated. OT infusions into the ACE have no effect on LQ but do significantly extend LD. This appears to be further confirmation of the general hypothesis that different brain sites may control different aspects of lordosis. Results from Experiment 4 indicate that the MPOA may primarily regulate lordosis frequency while the VMH may be responsible for other aspects of lordosis. It may now be possible to further refine this hypothesis: in the light of Experiments 4 and 5, it appears that the VMH serves as generator of this behavior, that the ACE controls lordosis duration, and that the MPOA controls lordosis frequency. This lordosis maintenance function of the ACE is consistent with the findings of Grossmann (1962) concerning the role of the amygdala in the maintenance of eating and drinking behaviors.

Anatomical considerations may provide some additional support for this hypothesis. It has been shown with tracing studies that the ventrolateral VMH projects to the ACE and the dorsomedial VMH to the MPOA (Saper et al. 1976), but the ACE does not project to the MPOA or directly to the VMH (Krettek and Price 1978), which suggests that lordosis
Figure 5. The figure shows the mean lordosis fraction (lordosis quotient/100) ± S. E. M. and the mean lordosis duration ± S. E. M. for various doses of oxytocin infused into the central nucleus of the amygdala in female rats treated subcutaneously with 3μg estradiol benzoate 48 h before testing and 150 μg progesterone 4-6 h before testing.
related signals may originate in the VMH. The hypothalamus is considered to be a neurohumoral transducer that converts neural signals into hormonal signals (Scharrer and Scharrer 1954). It may be equally useful to consider it a humoroneural transducer responding to humoral conditions with neural output. In particular, the VMH may respond to humoral events following estrogen and progesterone treatment with the generation of neural signals that result in lordosis behavior. These signals are then relayed to the MPOA and ACE for the incorporation of the relevant frequency and duration information, respectively.

SECTION III
Investigations of the receptor types involved in the generation of lordosis at central sites that concentrate radiolabelled estradiol and respond to oxytocin administration.

Although there appears to be only one OT receptor, OT can also interact with the primary vasopressin receptors due to the structural similarities between oxytocin and vasopressin (Antoni 1987, Cheng and North 1989, Manning and Sawyer 1989). There are two primary vasopressin receptor subtypes to which OT can bind: $V_1$ receptors mediate the vasopressor effects of vasopressin while $V_2$ receptors mediate the antidiuretic effects of vasopressin.
It is therefore important to investigate which receptors may be responsible for the effects of central OT infusions on lordosis, especially given the results of Experiments 1 - 4 where it became apparent that OT had both an inhibitory effect (Experiments 1 and 2) and a facilitatory effect (Experiment 4). Furthermore, lordosis can be mediated by different receptors at different sites.

The OT agonist [Thr4, Gly7] - oxytocin and antagonist [d(CH2)5, Tyr(OMe)2, Orn8] - vasotocin have activities at the various receptors as indicated in the table below (adapted from 41,43).

Table 1.

<table>
<thead>
<tr>
<th>receptor type</th>
<th>oxytocic</th>
<th>vasopressor (V1)</th>
<th>antidiuretic (V2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>agonist</td>
<td>(moderate +)</td>
<td>(very weak +)</td>
<td>(very weak +)</td>
</tr>
<tr>
<td>antagonist</td>
<td>(potent -)</td>
<td>(potent -)</td>
<td>(very weak +)</td>
</tr>
</tbody>
</table>

It should be clear from the table that the use of these two compounds may give some indication of which receptor mediates lordosis at a particular central site. If V2 receptors only are responsible for lordosis, then neither agonist nor antagonist should have an effect on lordosis. If V1 receptors exclusively mediate lordosis, then the
antagonist should have an effect but the agonist not. If the oxytocin receptor was solely responsible for the induction of lordosis, then the agonist and antagonist should have opposite effects on lordosis. However, there is some indication that both OT and V1 receptors are involved in lordosis behavior (Caldwell et al. 1990). In particular, it appears that in the MPOA of female rats chronically treated with estrogen, a uterotonic antagonist (OT receptor antagonist) inhibits lordosis while a V1 antagonist facilitates lordosis. It is not known what the time course of OT and V1 receptor activation by the antagonist is and it is difficult to make a prediction about results to be obtained from the concurrent blocking of OT and V1 receptors.

Experiment 6

The effects of the OT agonist [Thr4, Gly7] - oxytocin and antagonist [d(CH2)5, Tyr(OMe)2, Orn8] - vasotocin on lordosis when infused into the LV, MPOA and VMH of female rats treated with estrogen and progesterone.

Infusion of OT into the lateral ventricles of female rats treated with estrogen and progesterone can either facilitate (Arletti and Bertolini 1985, Gorzalka and Lester 1987, Caldwell et al. 1989) or inhibit lordosis (Experiments 1 and 2), an effect that may be dependent on OT concentration,
steroid regimen or receptor type activated. For instance, at low concentrations OT may either inactivate OT receptors or primarily bind to an inhibitory receptor, perhaps the V2 receptor. In the MPOA, a uterotonic antagonist blocked the facilitatory effects of OT infusions in female rats treated chronically with estrogen (Caldwell et al. 1990). Since the presence or absence of progesterone can have an effect on OT receptors (Schumacher et al. 1989, Schumacher et al. 1990) and OT-induced lordosis (Gorzalka and Lester 1987, Schumacher et al. 1989), it is important to confirm the results obtained by Caldwell and co-workers (Caldwell et al. 1990) in animals treated also with progesterone. Whether a uterotonic receptor is responsible for the induction of lordosis upon the infusion of OT into the VMH in a manner analogous to that in the MPOA (Caldwell et al. 1990) is not known, although it is known that estrogen induces these receptors in the VMH (de Kloet et al. 1986, Johnson et al. 1989) and that they are dispersed in response to progesterone (Schumacher et al. 1989, Schumacher et al. 1990). The receptor type responsible for lordosis behavior in response to infusions of OT into the LV, MPOA, and VMH of steroid-primed female rats was investigated in Experiment 6.

Materials and methods

Twenty Sprague-Dawley female rats were bilaterally ovariectomized and provided with chronic intracerebral
cannulae directed at the LV, MPOA and VMH according to procedures described in the General Method section. The oxytocin agonist \([\text{Thr}^4, \text{Gly}^7]\) – oxytocin and antagonist \([\text{d(CH}_2\text{)}^5, \text{Tyr(OMe)}^2, \text{Orn}^8]\) – vasotocin were obtained from Peninsula Laboratories Inc., Belmont, CA. These peptide analogues were dissolved in physiological saline a few hours prior to use and administered 30 minutes before behavioral testing of each animal. Two concentrations of agonist (100 ng and 500 ng) and two concentrations of antagonist (100 ng and 500 ng) as well as a saline control group were employed instead of the various oxytocin doses used in Experiment 4. Cannula placements were histologically verified and only the data from animals with correct intracranial cannula placements were analyzed. Due to attrition and the fact that the LV and VMH tests were conducted for three weeks only, the data from these groups were analyzed using the Mann-Whitney U-test while data from the MPOA tests were analyzed with the Wilcoxon signed ranks procedure. In all other respects, animals were assigned to groups and tested in a manner identical to that described for Experiment 4.

Results

LV

Relative to the saline control infusions, infusions of both the agonist and antagonist inhibited lordosis. An infusion of 100 ng agonist significantly inhibited LQ scores
(Mann-Whitney $U = 13; n_1, n_2 = 8; p < 0.05$) and an infusion of 500 ng antagonist also significantly inhibited LQs (Mann-Whitney $U = 11.5; n_1 = 8, n_2 = 9; p < 0.05$). The infusion of 500 ng agonist and 100 ng antagonist did not have a significant effect on lordosis quotients. Lordosis duration scores were not significantly affected by any agonist or antagonist dose.

**MPOA**

No significant effects of the infusion of agonist or antagonist into the MPOA of estrogen and progesterone primed female rats were obtained.

**VMH**

No significant effects on lordosis quotient and lordosis duration were observed in response to the infusion of any dose of agonist or antagonist.

All the results are given in Figure 6.

**General discussion**

Both the agonist and antagonist attenuated lordosis frequency when infused into the LV of female rats treated with estrogen and progesterone. Although the effect on LQ of an infusion of 500 ng agonist into the LV was apparently similar to that of a 100 ng infusion, the effect of the
Figure 6. The mean lordosis quotients ± S. E. M. for the oxytocin agonist, saline control, and oxytocin antagonist infusions into the LV (top panel), VMH (middle panel), and MPOA (bottom panel) are shown. All animals were treated subcutaneously with 3 μg estradiol benzoate 48 h before testing and 150 μg progesterone 4-6 h before testing.
FIGURE 6.

![Graph showing the effects of different doses of agonists and antagonists on a LQ/100 scale. The graph compares LV, VMH, and MPOA regions.](image)

- **Y-axis:** LQ / 100
- **X-axis:** Agonist, ng/rat, Control, Antagonist, ng/rat

Legend:
- **O---O** LV
- **△---△** VMH
- **□---□** MPOA
former was not statistically significant due to greater variance. The infusion of 100 ng antagonist had less of an effect on LQ than the 500 ng dose. LD was not affected by these manipulations. In the VMH, neither agonist nor antagonist had any significant effect on LQ or LD. This is likely due to the small sample sizes (n = 4-6) since the pattern of results generally mirrored those obtained in the LV. Data generated from infusions of agonist and antagonist into the MPOA are of poor quality and little use but do show that the agonist tended to elevate and the antagonist to suppress LQ scores.

Although LD scores in the VMH were much higher than those in the MPOA, a comparison between these two sites was not done for several reasons. Control LD levels for the VMH were significantly higher than control MPOA levels (independent t = 4.013, df = 8, p < 0.01). Since control LQ scores for the VMH (85 ± 8.66) were also much higher than control LQ scores for the MPOA (20 ± 14.37), the possibility remains that steroid manipulations of the two groups may not have been equivalent in spite of the fact that the same regimen was followed. It is also possible that rating discrepancies between two different experimenters may have produced this effect. Finally, the agonist and antagonist tended to have different trends in the two sites which renders a comparison of LQ and LD values between them difficult.
The inhibition produced by both the agonist and antagonist when administered to the LV was unexpected. These data are difficult to interpret. It has been shown that antagonism of \( V_1 \) receptors results in the elevation of LQ scores (Caldwell et al. 1990). If oxytocin receptors are located at postsynaptic sites and vasopressin receptors at presynaptic sites, then the antagonism of both \( V_1 \) and OT receptors should result in the depression of LQ scores. This happens because postsynaptic events are more 'downstream' in the neurotransmission pathway and blocking neurotransmission downstream will inhibit sexual behavior irrespective of an upstream facilitation of neurotransmission. The inhibition shown by the 500 ng dose of antagonist is compatible with the view that \( V_1 \) receptors are located on presynaptic sites.

The inhibition found in Experiments 1 and 3 could be considered to be, amongst other possibilities, due to activation of \( V_2 \) receptors located on oxytocin neurons. Activation of these receptors attenuates OT neurotransmission and these receptors may consequently act as OT autoreceptors. The agonist used in Experiment 6 has only a third the activity of the OT peptide at OT receptors (Lowbridge et al. 1976). This translates into an equivalent OT dose of about 33 ng in response to a 100 ng agonist infusion. However, in Experiment 3, a comparable dose of OT had no effect on LQ. Activity of the agonist at \( V_2 \) receptors are also 1000-fold weaker than that of OT at these
receptors. It is therefore difficult to reconcile the inhibition of LQ by the infusion of 100 ng of OT agonist with the hypothesis that the activation of V2 receptors located on OT neurons are responsible for such inhibition. Consequently, one of the other alternatives, especially the existence of an inhibitory site or the activation of other peptidergic systems should receive more credence.

SECTION IV

Some temporal factors in the effects of centrally administered oxytocin on lordosis.

Investigations of the oxytocinergic pathways involved in lordosis behavior frequently involve the central administration of OT or its analogues (Arletti and Bertolini 1985, Gorzalka and Lester 1987, Schumacher et al. 1989, Caldwell et al. 1990). It would therefore seem reasonable to apply these substances at a time when the maximum number of OT receptors are available. This appears to be at about 24 h after acute subcutaneous estrogen administration (Johnson et al. 1989). However, many studies employing the central administration of OT or its analogues have been conducted approximately 48 h after estradiol benzoate administration (e.g. Arletti and Bertolini 1985, Gorzalka and Lester 1987, Schumacher et al. 1989, Schulze and Gorzalka 1991) when levels of OT receptors may have declined appreciably (Johnson et al. 1989) although they may still be elevated
relative to control levels. The hypothesis that functional OT receptors already exist this soon (circa 24 h) after estrogen treatment has not been tested.

Experiment 7

Since the peptide oxytocin plays an important physiological role in parturition (Fuchs 1985) and lactation (Robinson 1986), is employed clinically (Dawood 1985) and has effects on many behaviors, including lordosis (reviews by Argiolas and Gessa 1991, Kovacs 1986), an understanding of its time dependency is essential. We consequently designed Experiment 7 to investigate the effects of the time since estrogen administration on the effects of oxytocin on lordosis behavior in female rats employing the same OT agonist and antagonist used in Experiment 6.

Materials and methods

Animals and surgery

Thirty-three female Sprague-Dawley rats, from Charles River Canada, Inc., Montreal, were bilaterally ovariectomized and cannulated as described in the General Method section. The coordinates used for cannulation in the left lateral ventricle were: 0.2 mm posterior, 1.8 mm lateral and 2.9 mm ventral. Cannula placements were verified
as described before and only data from animals with correct placements were retained for statistical analyses. Placement testing resulted in the exclusion of data from 14 animals from statistical analysis.

Following surgery, all animals were individually housed in standard laboratory wire mesh cages in a colony room maintained on a reversed 12 h light/dark cycle (lights off at 06h30) and at 50% relative humidity, 21°C with free access to food and water.

Drug procedures

Estradiol benzoate and progesterone (both from Sigma) were dissolved in warm peanut oil and administered subcutaneously. All animals received 3 μg estradiol benzoate in 0.05 cc of vehicle at 14h00 and 28 h before the behavioral test of the first group (this means that group one received estradiol benzoate 28 h before testing, but that group six received the steroid 48 h before testing) and 150 μg progesterone in 0.1 cc of vehicle 4 h before each group was tested. The oxytocin agonist [Thr4, Gly7] - oxytocin and antagonist [d(CH2)5, Tyr(OMe)2, Orn8] - vasotocin were obtained from Peninsula Laboratories Inc., Belmont, CA. These peptide analogues were dissolved in physiological saline a few hours prior to use and administered 30 minutes before behavioral testing of each
animal. Both agonist and antagonist concentrations were 125 ng/μl and a total dose of 500 ng per animal was infused. Angiotensin II (Sigma) was dissolved in physiological saline to a concentration of 1 μg/μl and given at a dose of 1 μg per animal. All infusions were performed at a flow rate of 4 μl/minute and the infusion needle was left in the cannula for an additional 30 s to allow dispersion of the peptide away from the infusion site.

Behavioral testing

Lordosis testing was conducted as described in the General Method section. Only lordosis frequency was measured. The experimental animals were randomly assigned to five groups of six animals each as well as a sixth group with only three animals. All groups were tested by a blind observer only once during the same 24 h period. The first group was tested at 18h00 and 28 h after estradiol benzoate administration. Thereafter, every four hours another group was tested until group six was tested at 14h00 and 48 h after estradiol benzoate administration. Exactly two weeks later, the animals were randomly reassigned to six groups and the testing procedure was repeated as described above.
Results and discussion

The results are illustrated in Figure 7. No statistical analyses were done due to the small number of subjects per group. An examination of Figure 7 strongly suggests rhythmic activity in the effects of oxytocin on lordosis. Interestingly, a circadian fluctuation is also manifest in the control scores. However, caution needs to be exercised in the interpretation of these data. Due to the elimination of data from several animals, some of the data points in Figure 7 represent single test scores. Paucity of data is particularly problematic at the 22h00, 10h00 and 14h00 time points. Unfortunately, these points are the ones of particular interest. At 22h00, it appears as if the agonist strongly facilitates lordosis while the antagonist and control scores are similar to each other. If such a facilitation indeed exists, a full 16 h before those generally reported in the literature (Arletti and Bertolini 1985, Gorzalka and Lester 1987, Schumacher et al. 1989, Schulze and Gorzalka 1991), it would be of interest because it may require researchers to modify their experimental procedures and/or theoretical positions regarding the effects of oxytocin on sexual behavior in female rats. For similar reasons the general decline in lordosis scores from high values at 06h00 to lower values at 10h00 may also be of interest. Control values seem to increase again after 10h00 while agonist and antagonist values continue to decline. If
Figure 7. Mean lordosis quotients for the oxytocin agonist, oxytocin antagonist, and saline control groups over a 24 h period starting 28 h after the subcutaneous administration of 3 µg estradiol benzoate. All groups received 150 µg progesterone 4 h and drug infusions 30 minutes before testing. Indicated on the same graph is the light-dark regimen followed.
these findings could be replicated, it would suggest that investigations of oxytocinergic actions on sexual behavior ought to be reconsidered. A follow-up experiment was undertaken to further investigate these findings.

Experiment 8

Experiment 8 was designed to test the effects of the OT agonist and antagonist at 22h00 and 14h00 and to test the effects of only the agonist at 10h00. These times were chosen because Experiment 7 yielded little data at these times and they were of particular interest as discussed above.

Materials and methods

Animals and surgery

The same animals used in Experiment 7 were also used in Experiment 8. Because some animals were eliminated from the statistical analysis as described in Experiment 7, an additional group of 13 animals were ovariectomized, cannulated and housed as described in Experiment 7.
Drugs

Drug procedures were identical to those in Experiment 7. Animals in the 22h00 test group received estradiol benzoate 32 h before testing, those in the 10h00 test group received the steroid 44 h before testing and those in the 14h00 group received estradiol benzoate 48 h before testing. Progesterone and the oxytocinergic drugs were administered 4 h before and 30 minutes before testing, respectively. Animals in the 22h00 and 14h00 groups received either the OT antagonist, agonist or saline vehicle and those in the 10h00 group received either the agonist or saline vehicle.

Behavioral testing

Behavioral testing was identical to that described in Experiment 7 with the exception that animals were tested at only three time points. One group of animals was tested at 22h00 and another at 14h00. The test at 22h00 was repeated a few weeks later with an additional group of animals to increase the sample sizes. This same group was also used a week later for testing at 10h00.

Results

22h00. The mean LQ ± S.E.M. for the agonist, antagonist and saline groups was, respectively: 76.7% ± 5.58; 21.7% ±
14.24; and 23.3% ± 8.43. These results suggest a facilitation of lordosis by the agonist when compared to the control group. An analysis of variance indicated a significant treatment effect (F(2,15) = 9.627, p < 0.01) and a follow-up (Tukey, p < 0.01) confirmed that the facilitation of lordosis by the agonist was significant.

10h00. Lordosis quotients (mean ± S.E.M.) for the agonist and saline vehicle control groups were, respectively, 35.0% ± 9.06 and 32.5% ± 10.65. These differences were not statistically significant.

14h00. No significant differences were found between the agonist, antagonist and saline groups at this time. LQs ± S.E.M. were, respectively: 65.0% ± 14.78; 55.0% ± 16.48; and 62.0% ± 19.08.

22h00 vs. 10h00. The agonist induced a significantly higher LQ at 22h00 than it did at 10h00 (independent samples, t = 3.593, df = 12, p < 0.01). As indicated above, the means ± S.E.M. were 76.7% ± 5.58 and 35.0% ± 9.06, respectively. The values for the saline control groups at these times were 23.3% ± 8.43 and 32.5% ± 10.65, respectively, and did not differ significantly. These results are illustrated in Figure 8.
Figure 8. The mean lordosis quotient ± S.E.M. is indicated for the oxytocin agonist and saline groups at 22h00 and 10h00. The oxytocin agonist induced a significantly higher LQ at 22h00 than it did at 10h00 (p < 0.01). The oxytocin agonist-induced levels of lordosis at 22h00 were also significantly higher than those of the control group at the same time (p < 0.01).
LORDOSIS QUOTIENT

TIME OF DAY

0.0 20.0 40.0 60.0 80.0 100.0

22:00

10:00

Agonist

Solvent

FIGURE 8.
22h00 vs 06h00 vs 10h00. Control scores at 06h00, 80.0% ± 7.07, were significantly higher than both the control scores at 22h00 (23.3% ± 8.43, independent t = 4.753, df = 8, p < 0.01) and those at 10h00 (32.5% ± 10.65, independent t = 2.94, df = 10, p < 0.02).

Discussion

The results of Experiment 8 confirmed the trend towards a facilitation of lordosis at 22h00 by the agonist as seen in Experiment 7. Also confirmed were the generally low levels of lordosis seen at 10h00 in response to the agonist and in the control group. At 14h00, however, the control scores were lower and the agonist scores higher than those observed in Experiment 7. The combined results of Experiments 7 and 8 are shown in Figure 9.

General discussion

The agonist used in this study facilitated lordosis in female rats as early as 32 h after priming with estradiol benzoate. This finding provides support for the hypothesis that functional OT receptors already exist circa 24 h after subcutaneous estrogen administration. At 44 h and 48 h after estradiol benzoate injections, no facilitation was seen. The antagonist used in this study did not inhibit lordosis at any of the time points tested. In addition to the length of
Figure 9. The combined data from Experiments 7 and 8 showing the mean lordosis quotients for the oxytocin agonist, the oxytocin antagonist, and saline control groups over a 24 h period starting 28 h after the subcutaneous administration of 3 μg estradiol benzoate. The 22h00 time point shows the mean lordosis quotient ± S.E.M. All groups received 150 μg progesterone 4 h and drug infusions 30 minutes before testing. Also indicated on the same graph is the light-dark regimen followed.
Figure 9.
time since estradiol benzoate injections, the time of day also appeared to affect the levels of sexual receptivity expressed by female rats as was mainly evident in the control group.

A significant increase in hypothalamic OT receptors occurs as early as 24 h after subcutaneous (silastic capsule implantation) estrogen administration to female rats (Johnson et al. 1989). If the activation of central OT receptors causes an increase in lordosis of female rats as suggested in the literature (Arletti and Bertolini 1985, Gorzalka and Lester 1987, Schumacher et al. 1989, Caldwell et al. 1990, Schulze and Gorzalka 1991), then a facilitation of lordosis may be seen as early as 24 h after subcutaneous estrogen administration. This hypothesis is given support by the finding of a significant facilitation of lordosis in response to the intraventricular infusion of an oxytocin agonist at 22h00 or 32 h after acute subcutaneous administration of estradiol benzoate. Since it appears that functional OT receptors exist centrally at this time, it is reasonable to conclude that the rate limiting step in the activation of the oxytocinergic pathway contributing to lordosis behavior is that of OT peptide synthesis. Submaximal levels of OT (levels below that required for the full activation of all possible OT receptors contributing to lordosis behavior) can be augmented from exogenous sources and should result in a facilitation of lordosis behavior.
Maximal levels of OT, on the other hand, would already take full advantage of all OT receptors and the administration of exogenous peptide will represent no gain to the system and hence no facilitation of lordosis would occur.

Lordosis levels at 02h00 and 06h00 are very high for all groups and may reflect the greater availability of endogenous oxytocin at these times. Since OT receptors are already available at 22h00 but high levels of lordosis are not seen at this time without the administration of exogenous hormone, it would seem that the rate determining step in the activation of oxytocinergic pathways involved in the expression of lordosis at this time point is the rate of peptide synthesis. However, an oxytocinergic pathway may not be the 'last common pathway' and the increase in diurnal sexual receptivity may reflect the activation of another pathway contributing to lordosis behavior. A prime candidate for such a pathway is that mediated by serotonin. It is known that serotonin type 2 receptors (5HT_2) facilitate lordosis in female rats (Gorzalka et al. 1990). Furthermore, 5HT_2 receptors show a circadian rhythm with peak numbers being expressed during the light phase (Moser and Redfern 1985) and thus coincident with the high levels of lordosis seen in Experiment 7 at 02h00 and 06h00.

The general decline in receptivity levels after 06h00 may reflect a decline in OT receptor levels as discussed
earlier. This may imply that, in contrast to the situation at 22h00, OT receptor levels are now the rate limiting factor and that, provided adequate levels of OT peptide exist, exogenous peptide or analogues would not elevate lordosis quotients. This would account for the present findings where little difference was observed between agonist and saline control scores at 10h00 and 14h00 in Experiment 8, but would conflict with several studies reporting a facilitation of lordosis by intraventricular OT administration at 14h00 (48 h after estrogen administration). If, prior to the decline of the OT receptor, there is a conformation change of the receptor from a high to a low affinity state accessible to peptide molecules but not to the OT agonist employed in this study, then the present findings may be reconciled with earlier work. For instance, it is known that the presence of Mg$^{2+}$ can influence the affinity of the OT receptor for OT (Antoni and Chadio 1989) as well as for the agonist (Lowbridge et al. 1976).

It is not clear why there is almost a doubling of LQ scores from 10h00 to 14h00. This increase is not statistically significant, perhaps due to the relatively small sample sizes used in our study. However, if at this time there is indeed a decline in OT receptor levels as suggested by other research (Johnson et al. 1989), the apparent increase in sexual receptivity seen to occur
between 10h00 and 14h00 may be due to the activation of a facilitatory or inactivation of an inhibitory non-oxytocinergic pathway. For instance, it is known that activity in the vasopressinergic system inhibits lordosis behavior (Sodersten et al. 1983) and that a pronounced daily rhythm exists in female rats with estrogen implants which is opposite to that of the daily vasopressin rhythm controlled by the suprachiasmatic nucleus (Hansen et al. 1979). This decline in receptor levels would imply that studies that investigated the effects of OT on lordosis behavior 48 h after estrogen administration did so at a time that was not optimal for this type of investigation. Furthermore, when this decline in central OT receptor levels is coupled with high endogenous OT levels, it would render the interpretation of much of the existing literature on the effects of OT on lordosis problematic.

Studies investigating the effects of oxytocin on lordosis behavior in the female rat have frequently ignored the possible influences of circadian rhythms (e.g. Arletti and Bertolini 1985, Gorzalka and Lester 1987, Schumacher et al. 1989, Caldwell et al. 1989, Caldwell et al. 1990, Schulze and Gorzalka 1991). Since many factors associated with reproductive behaviors in the rat clearly show circadian fluctuations, e.g. the onset and termination of estrus (Kuehn and Beach 1963), lordosis behavior in female rats with estrogen implants (Hansen et al. 1979), the
expression of serotonin receptors (Akiyoshi et al. 1989), serotonin binding (Wesemann et al. 1986) and serotonin turnover (Cohen and Wise 1988), and the release of luteinizing hormone releasing hormone (Ching 1982), the effects of OT on lordosis may be circadially modulated.

Evidence to indicate that circadian factors may be at work in the expression of lordosis is suggested primarily by the control groups of Experiments 7 and 8 (due to larger samples at the time points compared). Here it was found that lordosis scores during the light phase tended to be higher than those of the dark phase, in particular, lordosis levels at 06h00 (40 h after estradiol benzoate administration) were significantly elevated with respect to the levels at 18h00 (28 h after estradiol benzoate administration) and 10h00 (44 h after estradiol benzoate administration). These results contrast with those of Hansen et al. (1979) who found a pronounced circadian rhythm, with peak lordosis activity in the dark phase, in female rats with estrogen implants. It should be noted that the results reported here may reflect a transition phenomenon since the rhythm observed appears to shift from peak activity in the light phase toward increased activity in the dark phase. As pointed out before, this rise and decline in levels of receptivity may reflect the availability of OT receptors or those of serotonin receptors.
However, it cannot be concluded from the present data that the activity of the oxytocinergic system or systems involved in lordosis is dependent on the time of day. Several lines of research indicate that the central oxytocinergic systems may not show daily rhythmic activity. For example, although there is a daily fluctuation in the OT levels of cerebrospinal fluid (CSF) in monkeys (Perlow 1982), such variations do not occur in the rat (Mens et al. 1982) and furthermore, no diurnal variation occurs in the levels of oxytocin messenger RNA in the supraoptic and paraventricular nuclei of the hypothalamus (Burbach et al. 1988). In addition, the control of prolactin release by OT is not dependent on the time of day (Arey and Freeman 1989). On the other hand, there is evidence that the administration of oxytocin increases serotonin levels (Pfister and Muir 1989). This may then translate into circadian effects of OT on lordosis because of the mentioned circadian rhythm expressed by 5-HT$_2$ receptors (Moser and Redfern 1985). Thus, although the activation of the central oxytocinergic system(s) involved in lordosis behavior show no direct circadian variations, they may activate systems that are circadially rhythmic and in such an indirect manner cause lordosis behavior to show rhythmic effects. We believe, therefore, that studies investigating the sexual behavior of female rats should take into account the possible operation of circadian factors in their interpretation of the results.
Taken together, like the phenomenon of postpartum estrus, the oxytocinergic facilitation of lordosis may be dependent on both the time of day and the length of time since estrogen administration. It is well known that OT is important during parturition and that plasma levels of this hormone are elevated during and up to one hour after parturition (Evans et al. 1989). However, postpartum estrus does not occur until about 10 h after parturition or later, depending on the time of day of parturition (Gilbert et al. 1985). It appears that by this time plasma levels of OT had returned to normal (Evans et al. 1989). If OT did facilitate lordosis behavior and was also centrally available concomitantly with peripheral release, one would have expected peak levels of postpartum mating to occur within a short period after parturition. It is possible that no receptivity is seen shortly after parturition because central OT receptors, which require estrogen for induction (de Kloet et al. 1986), may not be present. Estrogen levels are low during pregnancy in the rat and start to rise shortly before parturition (Slotnick 1975). Estradiol, when administered by way of silastic capsule implantation, causes increased OT receptor binding in the ventromedial hypothalamus within 24 h and withdrawal results in a significant reduction in OT receptor binding within 24 h (Johnson et al. 1989). Furthermore, post-partum increases in oxytocin receptors occur in discrete brain regions (Gelhard et al. 1986). This supports the suggestion that mating is
seen only after the induction of central OT receptors and that a certain time interval is required for their induction. In addition, postpartum mating does not occur till roughly the beginning of the dark phase, a phenomenon that is clearly circadian. Whether this effect is due to activity of the serotonin system is unclear given the fact that 5-HT$_2$ receptors are primarily expressed during the light phase (Moser and Redfern 1985) and that estradiol can change the circadian rhythms of serotonin turnover expressed in certain brain areas (Cohen and Wise 1988). In the light of the existing literature and those results reported in this paper, we tentatively suggest that sexual receptivity in female rats is described by a model in which an interval timer is coupled to a circadian gating mechanism in a manner analogous to that of postpartum mating (Gilbert et al. 1985). In particular, we propose that the interval timer constitutes the process of oxytocin receptor (and perhaps peptide) synthesis activated by estrogen administration and that the circadian gating mechanism is another neurotransmitter system (possibly the serotonin or vasopressin system) that shows circadian rhythmicity and is coupled to the oxytocin system.

The present findings point to a serious need to investigate the time course of OT receptor induction and OT peptide synthesis in response to acute subcutaneous estrogen administration. For instance, there exists the possibility
that the time course of OT receptor induction and/or OT peptide synthesis may vary depending on the central site investigated. In a recent paper, Caldwell et al. (1989) found a facilitation of lordosis in response to OT administration to the medial preoptic area but not to the ventromedial hypothalamus. This finding, although subject to other interpretations (Schulze and Gorzalka 1991), may also be due to a difference in the time course of OT receptor decay at these two sites. Finally, such responses of the oxytocinergic system at particular central sites to estrogen administration may depend on both the time of day of testing and the time elapsed since estrogen administration as proposed in the preceding paragraph. Unless these issues are clarified, the interpretation of studies investigating the role of the oxytocinergic system in the control of lordosis will be difficult. One would therefore suggest that studies investigating the effects of OT and its analogues on lordosis behavior should consider both the time of day and the time since estrogen administration.

GENERAL DISCUSSION

The present series of experiments concerning the central effects of oxytocin on lordosis has yielded several findings. Oxytocin in low concentrations inhibits lordosis when administered to the lateral ventricle, but not third ventricle, of female rats primed with both estrogen and
progesterone. In similarly steroid-primed animals, oxytocin infusions into the MPOA enhance primarily lordosis frequency, infusions into the VMH increase primarily lordosis duration while infusions into the ACE extend lordosis duration but have no effect on lordosis frequency. The administration of a moderate oxytocic agonist to the left lateral ventricle inhibits LQ while a compound with potent anti-oxytocic effects and potent anti-vasopressor effects also inhibits LQ. Finally, the same oxytocin agonist administered to the left lateral ventricles of female rats increased lordosis frequency as early as 32 h after estrogen treatment but not 48 h later.

The findings obtained from the infusion of several different doses of oxytocin into various central sites point to the fact that no single brain site 'controls' lordosis behavior, but that several sites contribute toward the generation of different aspects of this behavior. In this regard, I have pointed out that the VMH may be the site where lordosis is permitted or initiated, the MPOA contributes frequency information to this behavior while the ACE may be responsible for the maintenance of the lordosis posture once initiated. This interpretation also suggests a hierarchy of the sites involved: it is likely that tactile stimuli arising from contact with the male induce the VMH to produce 'lordosis signals' which it then relays to the MPOA and ACE. These two nuclei then relay information to the MCG
where all incoming signals are integrated to produce the lordosis posture.

The results from Experiments 3 and 4 suggest that oxytocin may have effects at more than one central receptor type. In addition to the oxytocin receptor, oxytocin is known to bind to vasopressin V₁ and V₂ receptors (Lowbridge et al. 1976, Manning and Sawyer 1989). As discussed before, activity at V₂-like receptors located on oxytocin neurons inhibit oxytocin release (Cheng and North 1989) and may inhibit lordosis. Conversely, vasopressin binds to the oxytocin receptor with a pharmacologic activity about 3% that of oxytocin. Therefore, the administration of a moderately high dose of vasopressin can be considered to be equivalent to that of a low dose of oxytocin. Vasopressin inhibits lordosis (Sodersten et al. 1983) and may consequently do so by acting in the manner of a low dose of oxytocin (Experiment 3). A further complication is the fact that in the presence of Mg²⁺ the affinity of the OT receptor for OT and some OT agonists is increased (Antoni and Chadio 1989). It has also been established that in the presence of Li⁺ the oxytocin molecule changes its conformation to resemble that of vasopressin (Rholm et al. 1985). It is therefore possible that Mg²⁺ may cause a conformational change in the peptide and not the receptor. This may account for the fact that arginine vasopressin is a more potent agonist at OT receptors than oxytocin itself in the absence
of Mg\textsuperscript{2+} but less powerful in the presence of Mg\textsuperscript{2+} (Antoni and Chadio 1989). Unfortunately, the results from Experiment 6 do not allow an unambiguous interpretation of the results obtained in Experiments 3 and 4.

What does become clear from the results of experiments reported here and elsewhere, is that there may be a considerable amount of 'crosstalk' between the oxytocin and vasopressin systems. Some results, presented in Table 2, strongly imply that both the vasopressin and oxytocin systems play a role in the control of lordosis behavior.

Table 2

<table>
<thead>
<tr>
<th>Agent</th>
<th>Effect at receptor</th>
<th>Behavioral effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>OT</td>
<td>4 u/mg 4 u/mg 520 u/mg</td>
<td>depressed/elevated</td>
<td>a/b</td>
</tr>
<tr>
<td>AVP</td>
<td>369 u/mg 323 u/mg 14 u/mg</td>
<td>depressed</td>
<td>c</td>
</tr>
<tr>
<td>GT-OT</td>
<td>.01 u/mg .002 u/mg 166 u/mg</td>
<td>depressed</td>
<td>d</td>
</tr>
<tr>
<td>VT</td>
<td>pA = -7.96 .01 u/mg pA = -8.52</td>
<td>depressed</td>
<td>d</td>
</tr>
<tr>
<td>PM-OT</td>
<td>pA = -8.62 .31 u/mg pA = -6.62</td>
<td>antagonist depressed</td>
<td>e</td>
</tr>
<tr>
<td>dTM</td>
<td>pA = -8.62 .31 u/mg</td>
<td>elevated</td>
<td>e</td>
</tr>
</tbody>
</table>

OT = oxytocin, AVP = arginine vasopressin, GT-to = [Thr4, Gly7] - oxytocin, VT = [d(CH2)5, Tyr(OMe)2, Orn8] - vasotocin, PM-to = [Pen1, pMePhe2, Thr4, Orn8] - oxytocin, dTM = [d(CH2)5, Tyr(Me)] - AVP; a = Experiment 3, b =
If only one of the OT or V₁ receptor types mediated lordosis behavior, one would have expected to see similar effects on LQ upon their administration. Given the fact that opposite effects are obtained, one is lead to conclude that at least two systems with opposite actions mediate lordosis.

A possible way in which these two peptidergic systems may interact is the following. Oxytocin may act as an agonist at oxytocin receptors and as a partial agonist at vasopressin receptors (e.g. V₁ receptors), while vasopressin may act as an agonist at vasopressin receptors and as a partial agonist at oxytocin receptors. Low concentrations of oxytocin may then inhibit lordosis because there may be little effect at oxytocin receptors while the tonic inhibition of vasopressin would be enhanced, especially if oxytocin at low concentrations is more likely to bind at vasopressin receptors. High concentrations of oxytocin would activate the lordosis facilitating oxytocin system and tend to disrupt the lordosis inhibiting vasopressin system. Low concentrations of exogenous vasopressin would augment tonic vasopressin inhibition and have little effect at the OT system, while high concentrations of vasopressin would further activate the inhibitory vasopressin system and tend to disrupt the facilitatory oxytocin system. The net effect
of such a system would be that lordosis only occurs when high levels of oxytocin are present.

Finally, the results from Experiments 7 and 8 indicate that temporal factors should be taken into consideration when evaluating the effects of oxytocin on lordosis. The circadian rhythms expressed by the vasopressinergic and serotonergic systems (Mens et al. 1982) and the time course of activation of the oxytocinergic system (Experiment 8) may be important factors in the regulation of lordosis behavior given that interactions between these two systems can occur (see Section III). Since temporal factors are known to be important in the serotonergic and vasopressinergic systems, they could also extend to other neurotransmitter systems involved in lordosis. When administered into the LV, the oxytocin agonist employed elevated LQ scores with respect to control infusions as early as 32 h after estrogen injection. Unfortunately, lordosis duration scores have not been obtained and it is not certain whether LD would also show increases at this time. It would be interesting to establish whether the time course of functional oxytocin receptor induction by estrogen administration follows the same pattern in the MPOA, the VMH and the ACE.

Whether the effects of OT on lordosis are mediated by other neurotransmitter systems, e.g. the serotonin system, or whether OT mediates the effects on lordosis of other
neurotransmitter systems is not currently known. These experiments (reported in Sections III and IV) suggest that interactions do occur with other neurotransmitter systems and the oxytocinergic system may be only one of several neurotransmitter systems mediating lordosis. It is also possible that a specific aspect of lordosis behavior, such as dorsiflexion, is mediated by a specific neurotransmitter system, while a different aspect of lordosis, such as the maintenance of the lordosis posture, is mediated by another neurotransmitter system.

Aside from the clinical application of OT during labor and lactation, OT may have other potential clinical applications as well. It is known that OT plays a role in the development of drug tolerance (see Kovacs 1986 and Argiolas and Gessa 1991 for reviews) and that estrogen stimulated neurophysins (precursor molecules of oxytocin and vasopressin) are increased more than 100% in all psychiatric patients who recover satisfactorily from electroconvulsive therapy (Scott et al. 1986). As far as sexual behavior is concerned, OT concentrations in CSF rise shortly after ejaculation in male rats (Hughes et al. 1987), while intracerebroventricular infusions of OT in male rats produce repeated episodes of penile erections and yawning (Melis et al. 1989). In human females, those taking oral contraceptives showed increased oxytocin plasma levels during the menstrual cycle compared to those not taking oral
contraceptives (Uvnas-Moberg et al. 1989). Given the involvement of OT in reinforcement (see Kovacs 1986) and sexual function, and the possible involvement in emotional dysfunction (Scott et al. 1986), one could speculate that therapy aimed at the oxytocinergic system may address problems associated with both emotional and sexual dysfunction. It is furthermore tempting to conjecture that the effectiveness of lithium in the treatment of manic and bipolar depressed patients (Cooper et al. 1986) can be ascribed to its effects on the conformation of the OT molecule (Rholam et al. 1985) resulting in a modulation of oxytocinergic function. Whether any of the findings reported here will have clinical relevance, remains to be determined. It is expected though that the effect of OT dose on sexual behavior (Section I) and the temporal parameters of OT action (Section IV) may prove useful in any application of OT-based therapy.

Oxytocin has been shown to have manifold behavioral effects (see Kovacs 1986 and Argiolas and Gessa 1991 for reviews) and the results obtained in the experiments reported here need to be interpreted in the larger context of oxytocin's variety of behavioral effects. To put it more forcefully, if the central infusion of oxytocin can lead to one of several different behaviors, some of which may be mutually incompatible, how is a particular behavior selected for execution?
There is evidence indicating that independent oxytocinergic systems may exist, for instance, OT is released into the circulation from the posterior pituitary by paraventricular and supraoptic neurosecretory cells that do not project to other brain areas (Buijs et al. 1985) and it has been shown that levels of OT in the CSF and in blood are independently regulated (Robinson 1983). It is further supported by the fact that immunostaining of perivascular neurons in the preoptic region disappear by day two postpartum and that another population of oxytocinergic neurons, not associated with blood vessels, appear in these regions (Jirikowski et al. 1989). Furthermore, only 30% of OT neurons in the VMH concentrate radiolabelled estrogen (Morrell and Pfaff 1982) and in some brain regions, but not in others, OT receptors are modulated by estrogen (de Kloet et al. 1986).

Separate oxytocinergic systems may control separate behaviors and some of these behaviors may be estrogen dependent while others may not be. It has been demonstrated that OT induces maternal behaviors such as pup retrieval, pup licking and nest building (see Mena et al. 1985). Several studies also point to the possibility that maternal aggression may be regulated by OT. Mayer and Rosenblatt (1987) found that treatments known to reduce sensitization latencies to pups also lead to increased aggressiveness of
the female toward a novel male. Furthermore, suckling
stimulation by the pups, which releases OT (Mena et al.
1985), is essential for the initiation of male-directed
aggression in mice during the time immediately post partum
(Garland and Svare 1988). In rats, lesions of the
peripeduncular area of the lateral midbrain, a part of the
ascending milk-ejection pathway, diminished maternal
aggression (Hansen and Ferreira 1986). Although no direct
evidence exists to indicate control of maternal aggression
by OT, the available data are too provocative to ignore.
Exactly this creates a dilemma: it is not clear how maternal
aggression toward a novel male is compatible with copulation
with a novel male - given that both behaviors may be
elicited by OT.

Oxytocin appears to have effects on many behaviors. Some
of these behaviors and some components of some of these
behaviors may be incompatible. This necessitates
coordination among the various oxytocin systems. Such
coordination may be effected in a variety of ways: different
brain sites, different receptor types and receptor affinity
states, different levels of receptor populations and peptide
availability, and different time courses in receptor and
peptide synthesis (perhaps at different central sites) may
all play a role. Therefore, these factors need to be taken
into account in the study of the effects of oxytocin on
lordosis behavior.
REFERENCES


Uvnas-Moberg, K.; Sjogren, C.; Westlin, L.; Andersson, P. O.; Stock, S. 1989. Plasma levels of gastrin, somatostatin, VIP, insulin and oxytocin during the menstrual cycle in women (with and without oral contraceptives).


