*J. Physiol.* (1979), **288**, *pp.* 189–202
*With 8 text-figures*
*Printed in Great Britain*



# FACILITATION OF THE LORDOSIS REFLEX OF FEMALE RATS FROM THE VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS

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(*Received 26 May 1978*)

SUMMARY

1. Effects of electrical stimulation of hypothalamic ventromedial nucleus (v.m.n.) on the lordosis reflex of female rats were examined in ovariectomized and oestrogen-primed animals with chronically implanted electrodes.

2. Lordosis triggered either by manual cutaneous stimulation or by male mounting, was facilitated by electrical stimulation of the v.m.n.

3. A gradual increase in lordosis performance followed a relatively long period of stimulation; never less than 15 min and usually about 1 hr of stimulation was necessary for maximum facilitation. Following the termination of stimulation, the performance returned gradually to the control level during a 5–8 hr period.

4. The optimal frequency of stimulation was between 10 and 30 Hz. Threshold for effective facilitation was, on the average, 12·5 $\mu$A.

5. Stimulation tended to induce larger facilitation when applied to the lateral side of v.m.n.

6. Pre-treatment with oestrogen was necessary to obtain facilitation by v.m.n. stimulation. The threshold dosage of oestrogen was 2·5 $\mu$g per animal.

7. Stimulation was effective in adrenalectomized rats, in dexamethasone-primed animals, and in rats pre-treated with exogenous progesterone. Thus, adrenal progesterone release is not required for the v.m.n. facilitation of lordosis.

8. Medial preoptic stimulation with the same parameters suppressed the lordosis reflex.

9. The v.m.n. participates in the control of lordosis by a facilitatory output. The delay before facilitation implies that the v.m.n. is not in the direct reflex-arc for the execution of lordosis. Rather, a summation or interaction process with an unusually long time course is involved.

## INTRODUCTION

The lordosis reflex, dorsiflexion of the vertebral column, is an essential element of female copulatory behaviour in rodents. This response depends strongly on oestrogen levels in rats, and appears to be most stereotyped among the simpler aspects of female copulatory behaviour (see Pfaff, Diakow, Zigmond & Kow, 1974, for review). For the execution of lordosis supraspinal control is required, for section of the thoracic cord abolished the response (Kow, Montgomery & Pfaff, 1977). Sensitivity of lordosis to oestrogen is attributed to supraspinal structures, because

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responses remaining in the spinal animal have not shown any dependence on oestrogen (Pfaff *et al.* 1974).

There is good reason to suppose that the ventromedial nucleus of the hypothalamus (v.m.n.) is involved in the oestrogen-sensitive supraspinal circuitry for lordosis. Preferential uptake of radioactive oestradiol has been found in portions of the v.m.n. (Pfaff & Keiner, 1973). In female rats bearing lesions in the v.m.n., normal copulation does not occur (Kennedy, 1964; Kennedy & Mitra, 1963; Mathews & Edwards, 1977). Implants of oestrogen into the v.m.n. restored lordosis in ovariectomized rats (Barfield & Chen, 1977; Dörner, Döcke & Moustafa, 1968). Thus, induction of lordosis could be correlated with activation of certain v.m.n. neurones, in which resting discharge and responsitivity can be facilitated by oestrogen (Bueno & Pfaff, 1976).

While it has been demonstrated that electrical stimulation of several forebrain and hypothalamic structures disrupts lordosis (Malsbury & Pfaff, 1978; Moss, Paloutzian & Law, 1974), no systematic observations have been made on effects of v.m.n. stimulation. The present investigation provides the first evidence that electrical stimulation of an oestrogen-concentrating neuronal structure can facilitate the lordosis reflex.

## METHODS

*Animals.* This report is based on observations made on sixty-five Sprague-Dawley albino female rats. All animals were purchased from Hormone Assay Laboratories, Chicago, Illinois, and were ovariectomized at least 2 weeks before the beginning of experimentation. During the course of behavioural observation, they were housed singly in a controlled environment at 24 °C with a reversed 12 hr light–dark cycle (lights off at 9 a.m.). Free access to laboratory chow and water was allowed at all times.

*Preparation.* The animals weighed between 260 and 320 g at the time of surgery. They were anesthetized with intraperitoneal Nembutal (35 mg/kg body wt.), and their heads were secured in a Kopf stereotaxic frame such that the skull was level between the bregma and lambda. Craniotomy was made in the parietal area, and the dura was incised while preserving the sagittal sinus.

Monopolar electrodes were constructed from 10% iridium-platinum wire 178 $\mu$m thick, coated with Teflon except for the cut tip. Impedance of each electrode was 25–27 k$\Omega$ at 1 kHz. In forty-eight among sixty-five animals, implantation of the electrodes was made bilaterally, aiming for the rostral part of the v.m.n., with the stereotaxic coordinates: 2·6 mm posterior (P) to the bregma, 0·6 mm lateral (L) to the mid line and 8·5 mm deep (D) from the surface of the dura. In the remaining seventeen animals, electrodes were implanted bilaterally in either the medial preoptic area (nine rats), medial thalamic nuclei (four rats), or the cerebellar cortex. Stereotaxic co-ordinates for these sites were: (1) medial preoptic area: P 0·5, L 0·6, D 7·5; (2) medial thalamic nuclei: P 4·0, L 0·5, D 7·5; (3) cerebellar cortex: P 9·0, L 1·0, D 2·5. Indifferent electrodes were made from strands of uninsulated stainless-steel wire wrapped around jeweller's screws placed in the frontal bone. Electrodes were soldered to Amphenol pins, and fixed to the skull with dental cement.

*Stimulation and testing procedure.* Each animal received a subcutaneous injection of either 5 or 10 $\mu$g oestradiol benzoate in sesame oil on the day of the operation. Screening tests with bilateral stimulation of v.m.n. were made on day 4 (day of injection = day 0), on which the animals were recovered from the operation and displayed weak lordosis in response to manual cutaneous stimulation of the flanks followed by the rump-tail base-perineum region (Pfaff, Montgomery & Lewis, 1977). The varying dosage of oestrogen of 5 or 10 $\mu$g was found not to result in a significant difference either in the lordosis score or quotient, so the data are combined in appropriate categories of pre- and post-stimulation.

In the animals in which facilitation of lordosis was obtained in the screening, electrical stimulation was repeated under a variety of experimental conditions. Systematic analyses were

made on threshold dose of oestrogen for successful facilitation by electrical stimulation, and changes in effects of the stimulation were observed in relation to the time elapsed after a single injection of oestrogen. Possible involvement of adrenal progesterone in the facilitation of lordosis was investigated in animals which were adrenalectomized, had received dexamethasone (to fix ACTH levels at low, constant values), or were pre-treated with exogenous progesterone in addition to oestrogen. In the last case, animals which had a relatively low lordosis score with oestrogen treatment alone were selected.

For each test session, the animal was placed in a clear plastic cage. The lordosis performance of any animal at a given time was assessed by applying manual cutaneous stimuli 5 times, at brief intervals. Lordosis reflex strength upon each stimulus application was rated using a scale from zero (no vertebral dorsiflexion) to 3 (strongest possible response), and the average of five ratings was calculated. This averaged value, which we termed the lordosis reflex score, provided virtually identical results when collected independently by two investigators. Discrepancies larger than 0·3 were rarely found between our estimates on identical animals. Test-retest reliability was also high.

Control tests before electrical stimulation usually consisted of four or five determinations of the lordosis reflex score, separated by intervals of 5–10 min. Within 5 min after the completion of the last prestimulation tests, monopolar electrical stimulation of the brain was begun. The stimulation consisted of trains of balanced negative–positive biphasic square-waves produced by a circuit for constant current stimulation including two Grass S-44 stimulators and SIU-5 stimulus isolation units. The train of pulses was continued for 30 sec and interrupted for another 30 sec, repeated alternately for the duration of the electrical stimulation period (most stimulation lasted longer than 1 hr). At various times during electrical stimulation, usually at intervals of 5–15 min, lordosis reflex score was determined.

Within each session of electrical stimulation, current intensity, pulse frequency, pulse duration and the separation of square waves in each biphasic pulse pair were kept constant. Parameters of the stimulation were monitored on an oscilloscope. The most typical parameters of v.m.n.-stimulation were: frequency = 10 Hz; intensity = 50 $\mu$A; duration of each pulse = 0·2 msec; and separation of square pulses within each biphasic pair = 0·1 msec. In many cases, parameters of electrical stimulation were systematically varied between test sessions. Intensity of the stimulation is referred to in terms of the current per electrode of the leading, cathodal pulse of each biphasic pulse pair.

Some tests were conducted by using male rats instead of manual stimulation. For these tests, the female rat was placed, before v.m.n. stimulation, in a mating arena with a stud male rat, where she remained until mounted 10 times. This test was repeated immediately after the female rat had received v.m.n. stimulation. The behavioural measure used to evaluate v.m.n. stimulation effects in tests with male rats was the lordosis quotient (per cent lordosis occurrence per ten mounts).

*Data analysis.* The highest lordosis reflex score during v.m.n. stimulation was compared with that before v.m.n. stimulation. For summary statistics, the mean absolute increase and mean per cent increase in lordosis reflex score were calculated. The latency of the facilitation was defined as the time from the beginning of v.m.n. stimulation until the lordosis reflex score reached 90 % of the maximum value attained during v.m.n. stimulation. Threshold for the facilitation of lordosis was expressed in terms of the amplitude of the cathodal pulse.

The same summary statistics were made for tests which used male rats. In cases of decreased lordosis performance, such as during medial preoptic stimulation, the same procedures were used to assess the mean absolute decrease and per cent decrease. All scores were converted by arc-sine transformation and compared by the $t$ test.

*Histological analysis.* After all the tests were completed, some of the animals were used for another investigation, on the effects of v.m.n. lesions on lordosis (see the following paper, Pfaff & Sakuma, 1979). Following the completion of all observations, animals were anaesthetized with an overdose of Nembutal and were transcardially perfused with 10 % formalin. The brain was fixed in formalin and frozen serial sections (100 $\mu$m) were made in the frontal plane. The sections were stained with luxol fast blue and cresyl violet. In the animals with lesions, precise depth of the tip of the electrode was sometimes difficult to determine, but anterior–posterior and lateral placements of the electrode were precisely measured in all animals. With a microprojector, the distance of each electrode from the mid line was measured and used in the analysis of the relation between electrode placement and facilitation of lordosis.

RESULTS

Electrical stimulation of the v.m.n. facilitated lordosis in response to manual cutaneous stimulation ($P < 0{\cdot}001$) and in response to mounts by male rats ($P < 0{\cdot}01$), while stimulation of the medial preoptic area had the opposite effect (Fig. 1). In terms of per cent increase, the effect of v.m.n. stimulation ranged between 53·3 and 150 % of the pre-stimulation score. In contrast, animals with medial preoptic electrodes exhibited a significant decline both in terms of lordosis score ($P < 0{\cdot}001$) and lordosis quotient ($P < 0{\cdot}01$) following stimulation with parameters the same as those adopted for v.m.n. stimulation.

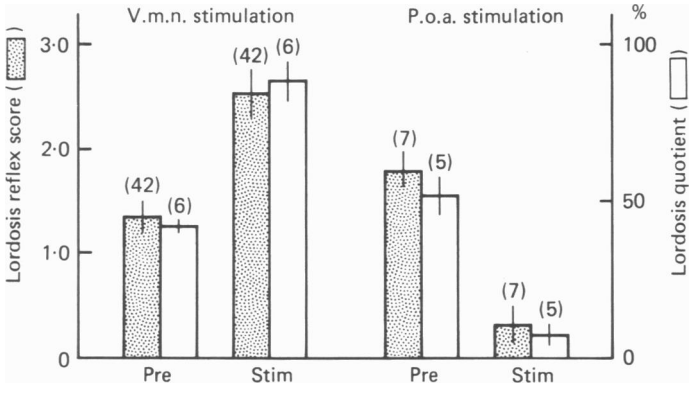


Fig. 1. Effects of electrical stimulation of the ventromedial nucleus (v.m.n.) and medial preoptic area (p.o.a.) on the lordosis reflex score (shaded columns) and lordosis quotient (open columns). Vertical bars represent S.E. of mean. Pre, prestimulation controls; Stim, lordosis performance during stimulation. Numbers in parentheses represent *n*.

*Time course of the facilitation*

In many rats, some facilitation was obvious 15 min following the onset of v.m.n. stimulation. However, it took as long as 1 hr to reach peak reflex performance in most tests. The score remained high during the course of stimulation. Termination of electrical stimulation did not result in an immediate disappearance of the facilitation. Gradual decline followed the end of v.m.n. stimulation, and the lordosis score resumed the level of the pre-stimulation control after 5–8 hr. Some examples of lordosis facilitation induced by v.m.n. stimulation of variable durations are shown in Fig. 2.

*Characteristics of effective stimulation*

Effects of unilateral stimulation were observed at thirty-four v.m.n. stimulation points in nineteen rats. Unilateral stimulation was effective in facilitating lordosis (Fig. 3).

The threshold of the facilitation of lordosis was on the average 12·5 $\mu$A and the response was graded. With increased stimulus intensity the response usually increased until it reached the maximum possible score at stimulus intensities 4 times threshold. However, variation in current intensity had no effect on the latency of the facilitation (Fig. 4).

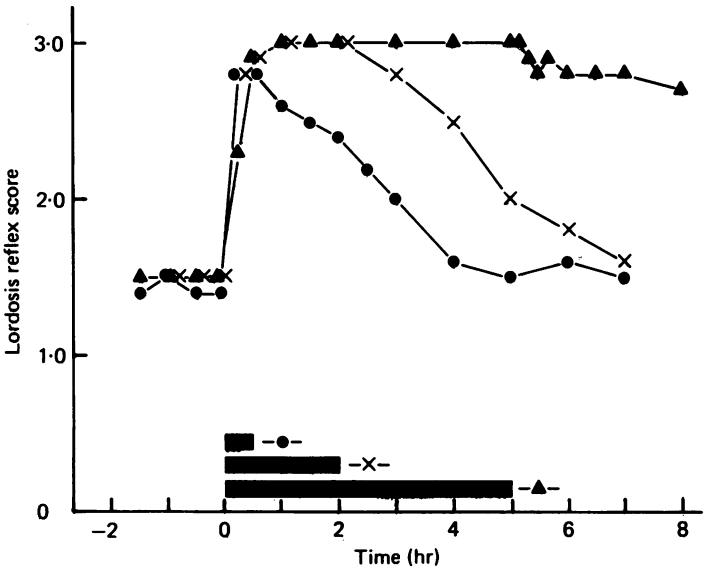


Fig. 2. Time course of facilitation of lordosis induced by electrical stimulation of ventromedial nucleus with different durations. Stimulation of v.m.n. was given for 0·5, 2, or 5 hr periods, as indicated by the bars at the bottom, with parameters: stimulus frequency 10 Hz, 50 $\mu$A per electrode, duration of leading (cathodal) pulse 0·2 msec.

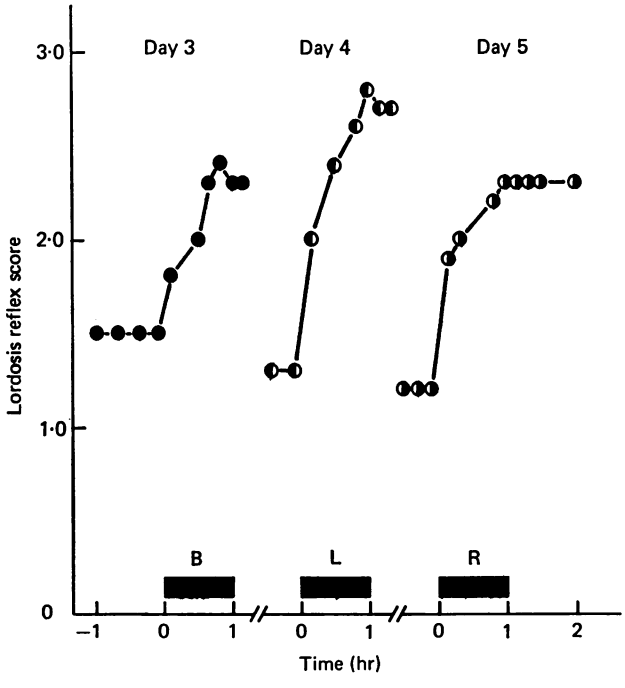


Fig. 3. Effects of bilateral (B) and unilateral (L, R) stimulation of ventromedial nucleus on lordosis in an individual rat. Stimulus intensity was 4 times threshold for each electrode arrangement.

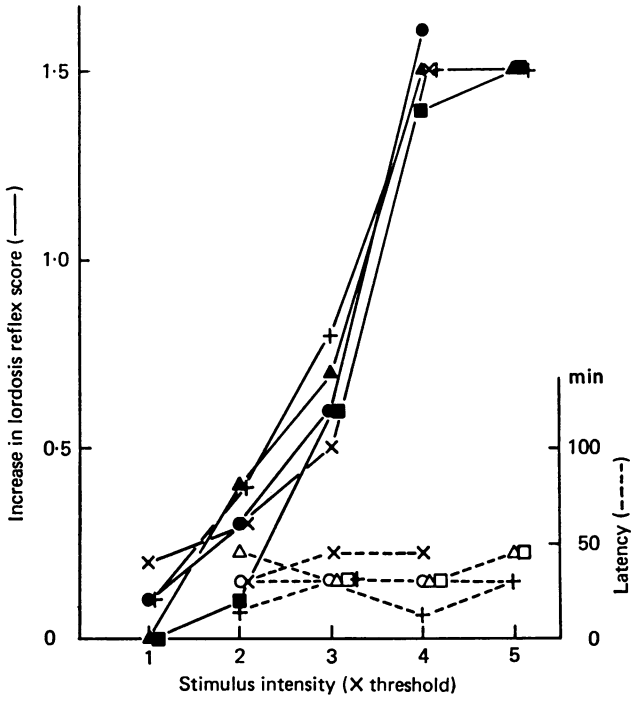


Fig. 4. Relations between intensity of v.m.n. stimulation, and magnitude of increase in lordosis score (continuous lines) or latency to 90% value of maximum response (dashed lines) from five representative experiments. Stimulus intensity is expressed as multiples of threshold current.

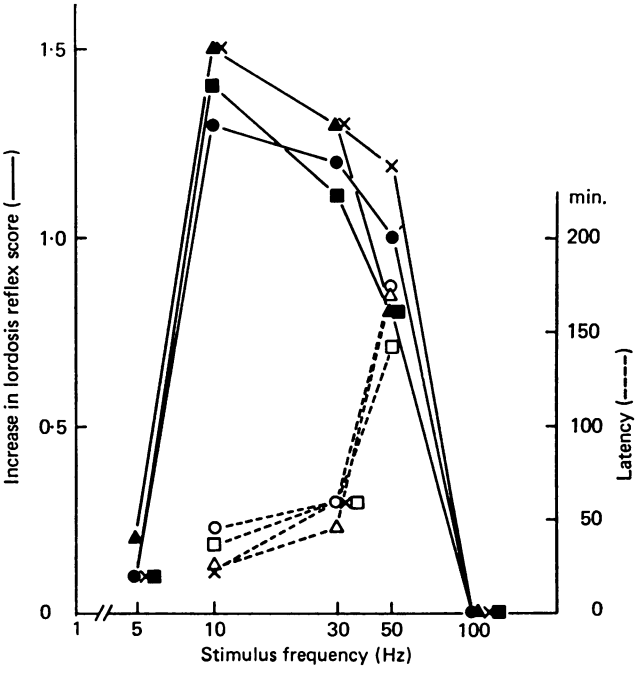


Fig. 5. Magnitude of increase in lordosis reflex score (continuous lines) or latency of facilitation (dashed lines), during v.m.n. stimulation at four times threshold intensity in four different animals, as a function of stimulus frequency.

The optimal stimulus frequencies for facilitating lordosis were between 10 and 30 Hz. Four representative experiments are shown in Fig. 5. At the more effective frequencies, the latency of facilitation was lower (Fig. 5).

Within the range of stimulus parameters used in this study, no abnormal behaviour was induced by v.m.n. stimulation. No abrupt change was seen in exploratory, feeding or drinking behaviour. In general, animals stayed calm without locomotion during the period of v.m.n. stimulation. At stimulus intensities over ten times lordosis facilitation threshold, signs of aversive effects began to appear. At these high intensities some animals showed chewing motions, or attempted to escape from the stimulation.

TABLE 1. Changes in pre- and post-stimulation values of lordosis reflex score following a single injection of oestradiol benzoate (OB) on day 0

| Dose of OB ($\mu$g) | | Lordosis reflex score (mean ± S.E. of mean) before (*pre*) and after (*post*) v.m.n. stimulation following a single injection of OB on day 0 | | | | | |
|---|---|---|---|---|---|---|---|
| | | Day 2/3 | Day 4/5 | Day 6/7 | Day 8/9 | Day 10/11 | Day 12/13 |
| 2.5 | *Pre* | 1·1 ± 0·5 (6) | 1·3 ± 0·2 (11) | 1·4 ± 0·1 (5) | 1·3 ± 0·1 (3) | 1·0 (2) | 0·5 (2) |
| | *Post* | 1·7 ± 0·9 | 2·4 ± 0·5* | 2·3 ± 0·4* | 2·0 ± 0·2 | 1·6 | 0·5 |
| 5 | *Pre* | 1·3 ± 0·1 (5) | 1·5 ± 0·1 (8) | 1·3 ± 0·1 (4) | 1·4 ± 0·2 (4) | 1·1 ± 0·1 (3) | 0·8 (2) |
| | *Post* | 2·2 ± 0·3** | 2·7 ± 0·1* | 2·3 ± 0·3** | 2·0 ± 0·5 | 1·6 ± 0·3 | 1·1 |
| 10 | *Pre* | 0·9 ± 0·5 (7) | 1·4 ± 0·3 (10) | 1·4 ± 0·2 (6) | 1·4 ± 0·3 (4) | 0·9 ± 0·2 (3) | 0·4 ± 0·2 (3) |
| | *Post* | 1·4 ± 0·5** | 2·7 ± 0·4* | 2·3 ± 0·3* | 2·1 ± 0·3 | 1·9 ± 0·2 | 0·7 ± 0·5 |

Post-stimulation value is significantly higher (* $P < 0·01$; ** $P < 0·05$) than pre-stimulation scores. Figures in parentheses denote $n$.

### *Effect of oestrogen on v.m.n.-stimulated lordosis facilitation*

Throughout this investigation, pre-treatment with oestrogen was necessary to elicit facilitation by v.m.n. stimulation. The effectiveness of the stimulation varied with the time elapsed after the oestrogen injection.

Statistical analyses were made on data collected in thirty-two animals which received stimulation on every other day following the administration of oestrogen (Table 1). It was seen that statistically significant lordosis facilitation was induced between day 2 and day 7, except in animals given 2·5 $\mu$g oestrogen, in which the stimulation on day 2–3 was ineffective. The largest v.m.n.-caused increase in lordosis performance was seen on day 4–5 following oestrogen, and the effect of v.m.n. stimulation decreased gradually or completely disappeared by day 12–13.

Since the largest v.m.n.-caused facilitation of lordosis was seen on day 4–5 after a single injection of oestrogen, the effects of v.m.n. stimulation were studied systemically on day 4 as a function of the dose of oestrogen (0·25–10 $\mu$g per animal). Experiments with each of five rats started with the smallest oestrogen dose and increased systematically with intervals of 18–20 days between experiments. As can be seen in Fig. 6, pre-stimulation lordosis performance reached a plateau with 2·5 $\mu$g oestrogen. The effect of v.m.n. stimulation on lordosis was dependent on the dose of oestrogen. A single injection of 2·5 $\mu$g oestradiol benzoate was the minimal dose to allow a significant increase in lordosis score by v.m.n. stimulation ($P < 0·05$).

*Histological analysis*

Of the forty-eight animals with bilateral implants, three had one electrode in the third ventricle. Thus, ninety-three stimulation sites were determined in histological sections and are shown in Fig. 7. Of the ninety-three electrode tips, seventy, in forty-two animals, were found within the nuclear boundary of v.m.n. Thirteen were in the area between v.m.n. and the hypothalamic arcuate nucleus or third ventricle. Four were found in the arcuate nucleus, and the remaining six located lateral or ventral to the v.m.n. No placements were found lateral to the fornix, or dorsal to the v.m.n.

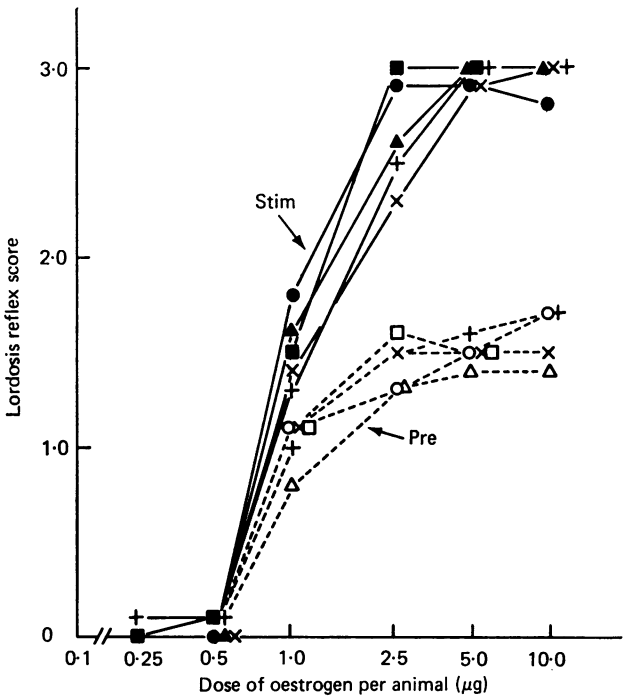


Fig. 6. Changes in the v.m.n.-stimulated lordosis reflex score (Stim) (continuous lines) or in the prestimulation control score (Pre) (dashed lines) as a function of oestradiol benzoate dose. Experiments were done 4 days after oestrogen injection. Stimulus parameters were the same as for Fig. 2, except that the duration was 1 hr. Significant differences ($P < 0{\cdot}05$) were seen between control and v.m.n.-stimulated lordosis in animals given at least 2·5 μg oestradiol benzoate.

Among these sites of implantation, thirty-four were stimulated unilaterally, in nineteen animals. These points are indicated in Fig. 7. Stimulation of the lateral part of the v.m.n. yielded larger increases in lordosis than stimulation in medial v.m.n. or the area medial to the nucleus. For each electrode used for unilateral stimulation, correlation between the stimulus-bound increase in the lordosis score and the straight line distance from the mid line was calculated. A significant correlation ($r = 0{\cdot}72$, $P < 0{\cdot}01$) was seen between the increase in lordosis and the distance from the mid line.

In nine animals with medial preoptic placements, all electrodes were found in

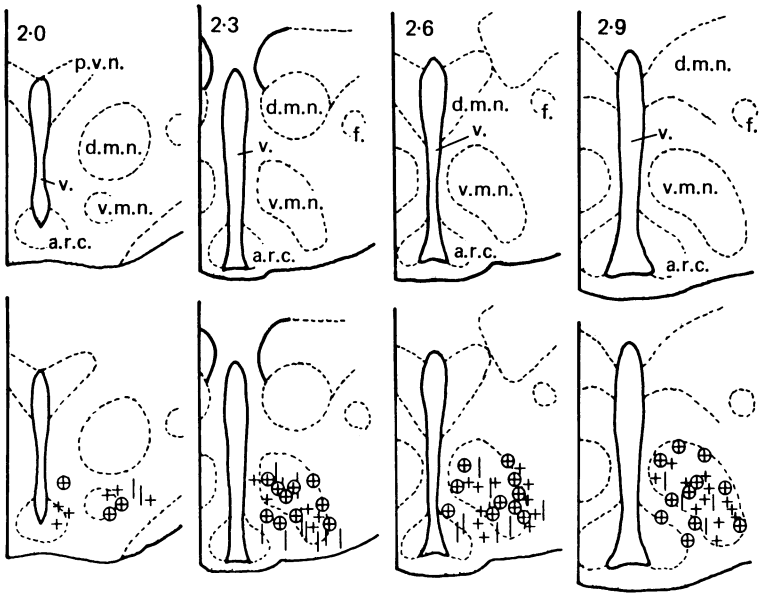


Fig. 7. Localization of stimulation sites in the ventromedial nucleus (v.m.n.) and adjacent hypothalamus ($n$ = 93). Points indicated by crosses (+) denote sites of electrode tips determined in animals without electrolytic lesions. Sites tested with unilateral stimulation ($n$ = 34) are encircled (⊕). Vertical lines represent the position of electrode tips in animals with lesions. Numbers in upper left corner of diagrams indicate the rostro-caudal distance in mm from the bregma. Abbreviations: a.r.c., hypothalamic arcuate nucleus; d.m.n., dorsomedial nucleus; f, fornix; p.v.n., paraventricular nucleus; v, third ventricle.

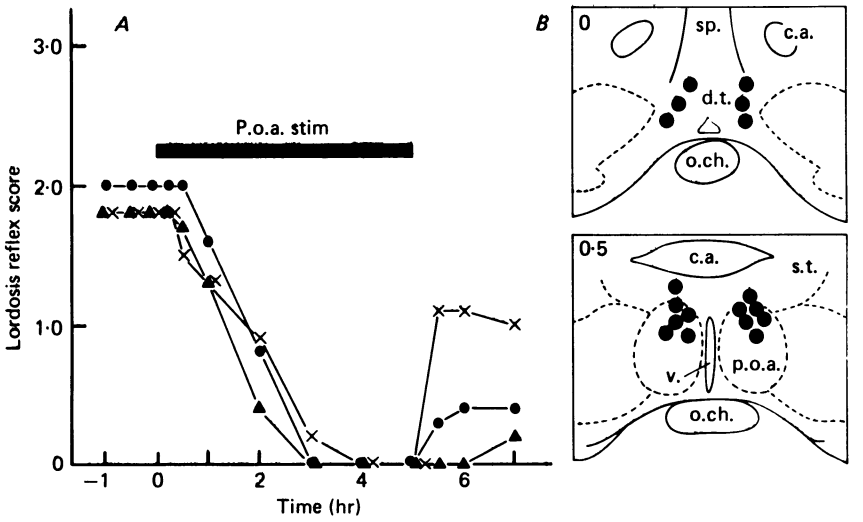


Fig. 8. *A*, representative examples of lordosis inhibition induced by electrical stimulation at preoptic levels. Stimulation was given bilaterally with the same parameters used in Fig. 2. *B*, histological diagrams showing the sites of effective stimulation. Numbers in upper left corner indicate the distance from the bregma in mm. Abbreviations: c.a., anterior commisure; d.t., diagonal tract of Broca; o.ch., optic chiasm; p.o.a., medial preoptic area; sp., septum; s.t., bed nucleus of stria terminalis; v, third ventricle.

either the rostral-most part of the medial preoptic area or in the diagonal band of Broca. Examples of typical inhibition of lordosis and effective stimulation sites are shown in Fig. 8. The time course of the inhibition was slow, and in that sense similar to that seen in the facilitation by v.m.n. stimulation.

An additional eight animals with electrodes implanted in the medial nuclei of the thalamus or in the cerebellar cortex also responded with an inhibition of lordosis when stimulated. The degree of inhibition varied between animals, but not one showed facilitation of lordosis during electrical stimulation.

### *Ruling out adrenal mediation*

Three lines of evidence indicate that the facilitation of the lordosis reflex results from direct activation of neural tissue and is not dependent on adrenal progesterone release secondary to the stimulation.

First, the facilitatory response was reproduced in four animals after adrenalectomy. No substantial difference was observed in the magnitude and time course of the facilitation before and after the operation. Secondly, blockage of ACTH increases by treatment with dexamethasone (5 mg per animal) 5 hr before v.m.n. stimulation failed to block the facilitatory effect of stimulation. Finally, v.m.n. stimulation potentiated lordosis in two animals which already had received 1 mg progesterone in addition to oestrogen, in which further (adrenal) progesterone release would not be effective in increasing lordosis strength.

## DISCUSSION

*Technical considerations.* The present results indicate that v.m.n. stimulation facilitates the lordosis reflex in oestrogen-primed ovariectomized rats. Stimulation with similar parameters in the medial preoptic area, thalamus, or cerebellar cortex resulted in an inhibition of lordosis, in confirmation of observations in the rat (Moss *et al.* 1974) and hamster (Malsbury & Pfaff, 1978; Zasorin, Malsbury & Pfaff, 1975). This evidence argues for an anatomically specific action of the electrical stimulation in these experiments. Results obtained in animals with controlled levels of progesterone showed that the stimulation effects were not mediated by the pituitary-adrenal axis, a point considered carefully because progesterone enhances lordosis in oestrogen-treated rats (see Young, 1961, for review).

Effective v.m.n. stimulation in the present study used low frequencies and low current values. Harris, Manabe & Ruf (1969) used biphasic pulses at a frequency of 50 Hz, with a pulse duration of 3 msec and pulse amplitude of 250–500 $\mu$A as optimal stimuli for induction of milk-ejection response by stimulation of the pituitary stalk. Everett (1965) induced ovulation with medial preoptic stimulation at a frequency of 30 Hz, with a pulse duration of 1 msec and amplitude of 500 $\mu$A. Fink & Jamieson (1976) and Jamieson & Fink (1976) found that optimal parameters for release of luteinizing hormone by preoptic stimulation in male rats were 60 Hz, 1 msec duration and 500 $\mu$A. The same stimulus was effective for causing secretion of luteinizing hormone releasing hormone (LH–RH) in female rats (Chiappa, Fink & Sherwood, 1977). When compared with these experiments, facilitation of lordosis was accomplished with relatively low electrical energy in the present study. Together

with the restricted localization of effective loci in the v.m.n., this suggests that spread of current was not a problem in this study.

The necessity of the long-lasting stimulation in the present study may have some relevance to the observation that for the induction of ovulation by electrical stimulation of the hypothalamus, duration is the critical parameter (Dyer, Mayes, Ter Haar & Yates, 1978). For the induction of ovulation, it remains unclear whether the long-lasting stimulation is required in order to elicit discharge of LH–RH from the hypothalamus (Dyer *et al.* 1978), or to potentiate adenohypophyseal response by a priming effect of LH–RH (Aiyer, Chiappa & Fink, 1974). However, possible mediation of v.m.n. facilitation of lordosis by LH–RH should not be overlooked, since this peptide is known to promote lordosis in oestrogen-treated rats (Moss & McCann, 1973; Pfaff, 1973), and the v.m.n. is considered to be one of brain structures which contain LH–RH cells (see Zimmerman, 1976, for review).

Wakerley & Lincoln (1973) have demonstrated in the hypothalamo-neurohypophyseal system that optimal frequency for stimulation is related to the rate of resting discharge of neurones in the system. This may explain the effectiveness of electrical stimulation with the low frequency of 10 Hz in this study, since the majority of v.m.n. neurones have been seen to have a rate of resting discharge less than 4 Hz in oestrogen-treated ovariectomized rats (Bueno & Pfaff, 1976).

Effects attributed here to neurones in and around v.m.n. were probably not due to fibres of passage. Demonstration of inhibitory effects of the medial preoptic stimulation excludes the possibility that descending projections from this structure could be involved in the facilitatory effect. In the anterior hypothalamus, neither electrical stimulation (Moss *et al.* 1974) nor lesion (Brown-Grant & Raisman, 1977) yielded significant effects on lordosis. These two structures are major sources of descending fibres which travel caudally near v.m.n. (Conrad & Pfaff, 1976*a*, *b*). It has been shown that 41 % of medial preoptic and anterior hypothalamic neurones were antidromically driven by stimulation of the v.m.n.-arcuate region (Dyer, 1973). Moreover, ascending projections from v.m.n. probably are not crucial for the effects on lordosis. These projections, demonstrated by anatomical (Krieger, Morrell & Pfaff, 1978) and electrophysiological (Dyer & Cross, 1972; Dyer, MacLeod & Ellendorff, 1976; Renaud, 1977) means, terminate (among other places) in the medial preoptic area and anterior hypothalamus and provide facilitatory inputs to these structures (Dyer, 1973). However, since the roles of these structures in lordosis are not strongly facilitatory, the ascending v.m.n. projections to them are probably more important for other behaviour patterns or neuroendocrine phenomena. Taken together, these arguments suggest that the stimulation effects on lordosis were mediated by projections descending from neurones in and around v.m.n.

*Physiological considerations.* Long-term treatment with oestrogen of ovariectomized female rats resulted in an increase in the number of active neurones in the v.m.n. with low discharge rates (Bueno & Pfaff, 1976). Oestrogen has accelerating effects on resting discharges of neurones in the medial anterior hypothalamus (Dyer, Pritchett & Cross, 1972). In contrast, in the medial preoptic area, Whitehead & Ruf (1974) and Yagi (1973) found units which decreased their resting discharge rates in response to oestrogen administration. Lincoln (1967) also noted that long-

term treatment with oestrogen of ovariectomized rats was followed by lower discharge rate of preoptic units.

Behavioural experiments also contrast v.m.n. with the preoptic area. Lesions of v.m.n. disrupt copulatory behaviour of female rats (Kennedy, 1964; Kennedy & Mitra, 1963), while lesions restricted to the preoptic region enhance lordosis (Powers & Valenstein, 1972). The differences in the effects between v.m.n. and medial preoptic area by lesions or electrical stimulation may reflect differences in the neuronal mechanism for lordosis, controlled by oestrogen under physiological conditions, as shown by the recording experiments.

It has been demonstrated that, in general, preoptic and hypothalamic neurones tend to fire at a low frequency (Dyer *et al.* 1972; Lincoln, 1967; Moss & Law, 1971), too slowly to account for lordosis reflex latencies. Moreover, most of these neurones do not respond specifically, strongly or quickly to somatosensory stimuli which are sufficient for triggering lordosis (Bueno & Pfaff, 1976). Therefore, v.m.n. neurones are unlikely to be involved directly in the reflex-arc for lordosis. Rather, they probably participate in lordosis control by exerting a tonic, hormone-sensitive bias on reflex arcs completed in the mid-brain or lower brainstem. The long time course required for full facilitation by v.m.n. stimulation supports this view and may reflect an unusually slow summation or interaction process.

We are grateful to Nicholas Brodyn for his technical assistance and to Gabriele Zummer for help in preparation of the manuscript.

This research was supported by N.I.H. grant HD-05751 and by an institutional grant from the Rockefeller Foundation for the study of reproductive biology.

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