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DEFICIT IN THE LORDOSIS REFLEX OF FEMALE RATS CAUSED BY LESIONS IN THE VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS

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SUMMARY

1. The effect of electrolytic lesions of the ventromedial nucleus of the hypothalamus (v.m.n.) on the lordosis reflex has been investigated in ovariectomized female rats. Lesions were made through chronically implanted platinum-iridium electrodes.

2. V.m.n. lesions did not disrupt lordosis immediately, but induced a gradual decline in the reflex. Lordosis performance reached its minimum no less than 12 hr after the lesion, and typically after 36–60 hr.

3. The magnitude of the lordosis deficit was related to the amount of v.m.n. damage. Destruction of other hypothalamic regions was without appreciable relation to the deficit. Within v.m.n., lesion size in the lateral, but not medial portion was significantly correlated with lordosis deficit.

4. Because of the slow time courses of v.m.n. lesions and stimulation (Pfaff & Sakuma, 1978) effects, it is postulated that the v.m.n. is not part of the direct reflex-arc for lordosis. Rather, neurones in v.m.n. are likely to exert a tonic hormone-dependent bias on brain stem reflex paths for this behaviour.

INTRODUCTION

Pfaff & Sakuma (1978) have demonstrated facilitation of the lordosis reflex of female rats by electrical stimulation of the ventromedial nucleus of the hypothalamus (v.m.n.). In ovariectomized female rats (Kennedy, 1964; Mathews & Edwards, 1977), hamsters (Malsbury, Kow & Pfaff, 1977), and guinea-pigs (Goy & Phoenix, 1963), tested following oestrogen treatment, animals with lesions in the neighbourhood of v.m.n. showed poorer lordosis performance. The present paper reports on effects on lordosis of small lesions in and around the v.m.n., the aim being to characterize more closely the hypothalamic substrate and the nature of its contribution. The time course of reflex loss is of particular interest in light of the electrical stimulation study (Pfaff & Sakuma, 1978), in which long term v.m.n. stimulation was required for facilitation of lordosis.

METHODS

Animals. Animals and procedures for the implantation of electrodes were described in the preceding paper (Pfaff & Sakuma, 1978). Electrolytic lesions were made in twenty-eight animals, which received daily injections of 5 μg oestradiol benzoate (subcutaneously in oil) for at least 14

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days before lesion. This dosage was chosen to allow lordosis without the necessity of progesterone injections, and all rats performed strong lordosis reflexes immediately before placement of lesions.

The general condition of the animal, such as responsiveness to somatosensory stimuli, was checked at several times during the behavioural observations.

Placement of the lesion. Lesions were made by passing DC anodal current of 1·0 mA for 15 sec through a chronically implanted platinum-iridium electrode in the v.m.n. A rectal electrode was used as cathode. For the observation of immediate effects of the lesion, unanesthetized rats were put in a restrainer and the current was passed. Lesions were made separately on each side, unless otherwise noted. In general, rats remained calm throughout the 15 sec period of lesioning No indication of pain was present.

Testing procedure and data analysis. Lordosis was induced by manual cutaneous stimulation of the flanks followed by pressure on the rump–tailbase–perineum region (Pfaff, Montgomery & Lewis, 1977). Lordosis reflex responses were rated using a scale explained in the preceding paper. The strength was rated from zero (no response) to 3 (strongest possible reflex). Stimulus applications were in blocks of five, and an average of the five ratings is referred to as the mean lordosis reflex score. Observations were made daily, and the stability of reflex performance verified for at least three days before the placement of the lesion. Following lesion, manual cutaneous stimuli were applied at intervals of 2 hr on the day of the lesion (day 0); 4 hr on days 1–3; and on day 4 and subsequently, reflex tests were made once daily.

In seven animals, supplemental tests were conducted using male rats. On each day after completion of manual stimulation tests, these females were placed with a stud male rat until mounted 10 times. These tests were made once daily before and after the lesion, and the lordosis quotient (per cent lordosis occurrence per ten mounts) calculated for each test.

All reflex scores and lordosis quotients were converted by arc-sine transformation, and pre- *vs.* post-lesion results compared by *t* test. For convenience of description, lesioned animals were divided into three groups according to their minimal post-lesion lordosis reflex score: severe deficit (lordosis score smaller than 0·5), moderate (0·6–1·0), or partial deficit (1·1–1·5) groups.

Histological analysis. Behavioural observations were discontinued approximately 4 weeks after the lesion. Animals were given an I.P. overdose of Nembutal, and transcardially perfused with 10% formalin. Serial frontal frozen sections (100 μm) were stained with luxol fast blue and cresyl violet and examined for the placement and extent of lesions. For each section through the lesion, drawings were made with the aid of a microscopic projector. The extent of each lesion was assessed by summing the weight of cutouts of the area of destruction from every drawing. Estimations were made separately for lesions within v.m.n. and those involving other structures. Additionally, the v.m.n. was divided into medial and lateral halves, and per cent damage calculated for each subdivision. Correlations between the lowest lordosis score after the lesion and per cent damage in medial or lateral v.m.n. were assessed by analysis of variance.

RESULTS

Immediately after the lesion and during the following period of observation up to 4 weeks, no obvious impairment was seen in motor co-ordination; control of posture and movement appeared normal. Corneal, grasp, and placing reflexes were normal. Animals with v.m.n. lesions showed exaggerated responses to somatosensory stimuli on the anterior part of the body. They tried to bite the stimulating objects. These responses were first noticed on day 1 and persisted as long as 3 weeks.

Deficit in lordosis

When tested within 5 min after the placement of the v.m.n. lesion, all rats showed strong and rapid lordosis reflexes in response to manual cutaneous stimulation, with no apparent change in reflex strength. Mounting by male rats also induced good lordosis at this time. No significant differences from pre-operative lordosis quotients were seen in animals tested with male rats within 2 hr after v.m.n. lesion.

Noticeable decreases in the performance of lordosis were first seen about 3–6 hr

after the placement of lesions in v.m.n. and reflex performance gradually declined during the following period (Fig. 1). No animal reached its minimal reflex score within 12 hr following the placement of the lesion (Fig. 2). The animals which showed the most severe deficits had shorter latencies to minimal performance than the ones with moderate or partial deficits.

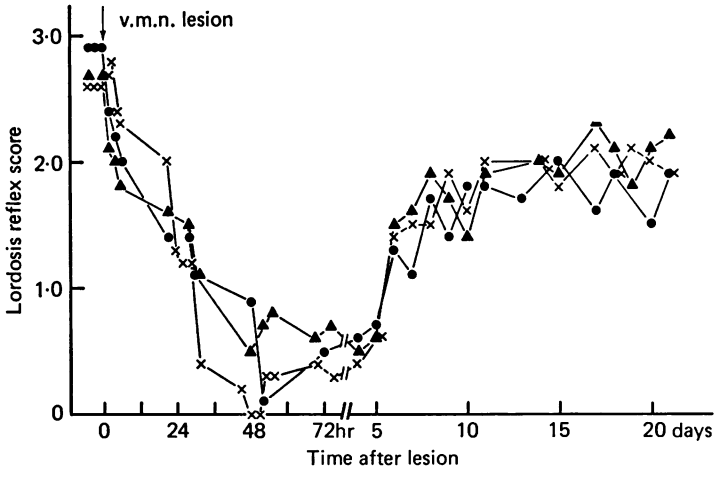

Fig. 1. Effect on lordosis of bilateral lesion of the ventromedial nucleus in three representative experiments. Each rat received daily injection of oestrogen throughout the period shown.

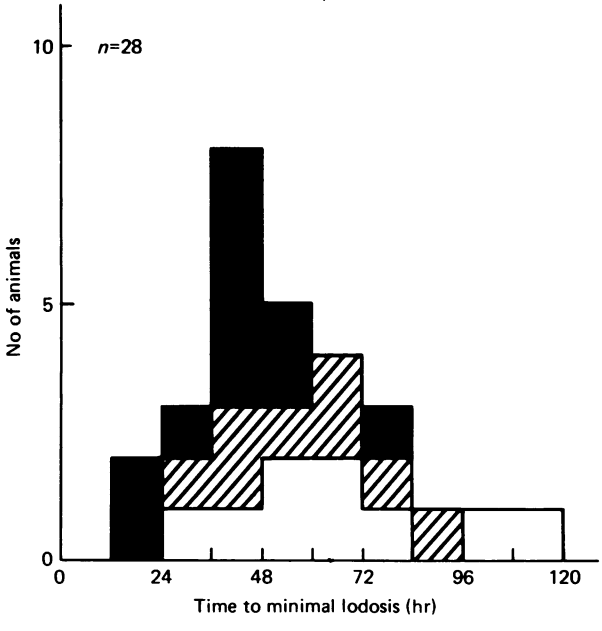

Fig. 2. Distribution of latencies (hr) to the minimal lordosis score, following ventromedial hypothalamic lesions. The black area depicts the animals with severe deficits (lordosis score less than 0·5); the hatched and open blocks indicate the animals with moderate (lordosis less than 1·0) and partial (less than 1·5) deficits, respectively.

At the time of lowest reflex performance, both the lordosis score to manual stimulation ($P < 0{\cdot}001$) and the lordosis quotient in response to mounts by male ($P < 0{\cdot}001$) were significantly lower than in the pre-lesion control period (Fig. 3). In general these two measures for the assessment of lordosis were well correlated with each other ($r = 0{\cdot}85$; $P < 0{\cdot}01$).

Following the time of minimal lordosis reflex performance, every animal showed some recovery. On the 14th day post-lesion, the recovery was significant either in lordosis reflex score ($P < 0{\cdot}01$) or lordosis quotient ($P < 0{\cdot}01$) when compared

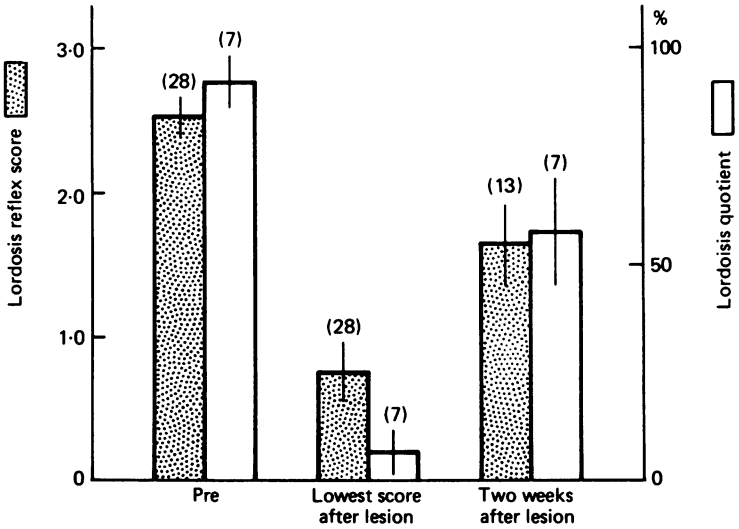

Fig. 3. Histogram showing lordosis reflex scores and lordosis quotients (mean ± S.E. of mean) for pre-lesion control, the minimal performance after lesion, and the data collected on day 14 following lesion. Shaded bars represent lordosis reflex score to manual stimulation and open bars represent lordosis quotients. Numbers in parentheses at top of each bar represent n.

with the lowest post-lesion scores. However, these values never reached those of the pre-lesion control period, nor was any further recovery seen during the period of observation up to 4 weeks. Thus the highest reflex scores ($P < 0{\cdot}001$) or lordosis quotients ($P < 0{\cdot}05$) after lesion were still significantly smaller than those obtained before lesion.

Histological analysis

The extent of the lesion was measured, and mean damage in v.m.n. and in other hypothalamic areas calculated for animals grouped according to magnitude of deficit in lordosis (Table 1). In animals with severe lordosis deficits, per cent loss of the v.m.n. ranged between 46·0 and 67·6%, with a mean of 55·4%. V.m.n. damage in animals with severe deficit in lordosis performance was significantly greater than in those with moderate deficit ($P < 0{\cdot}01$) or partial lordosis deficit ($P < 0{\cdot}01$). There were no significant differences among the three groups of animals in the extent of hypothalamic destruction outside the boundary of v.m.n.

In Fig. 4, examples are shown of the largest and smallest lesions in v.m.n. for each group of animals according to degree of lordosis deficit, and lesions in animals

with complete loss of lordosis are shown separately. In several animals, destruction included a portion of the caudal-most anterior hypothalamus. In some animals, parts of the hypothalamic arcuate or dorsomedial nuclei were also included in the lesion.

TABLE 1. Extent of damage in the hypothalamus, according to degree of deficit in lordosis.

Lordosis performance after v.m.n. lesion	Extent of hypothalamic destruction outside the v.m.n.	Extent of damage within the v.m.n.	Mean % loss of the v.m.n.
Severe deficit (11)	653·0 ± 275·1	282·0 ± 40·8	55·4
Moderate deficit (8)	662·8 ± 174·2	192·3 ± 36·7*	38·5*
Partial deficit (9)	559·9 ± 171·9	125·4 ± 77·0*	25·1*

Extent of lesions is indicated by weight of the paper (mg) clipped out from drawings along the boundary of each lesion. Numbers in parentheses denote *n*.

* Significantly smaller than damage in severe deficit group ($P < 0{\cdot}01$).

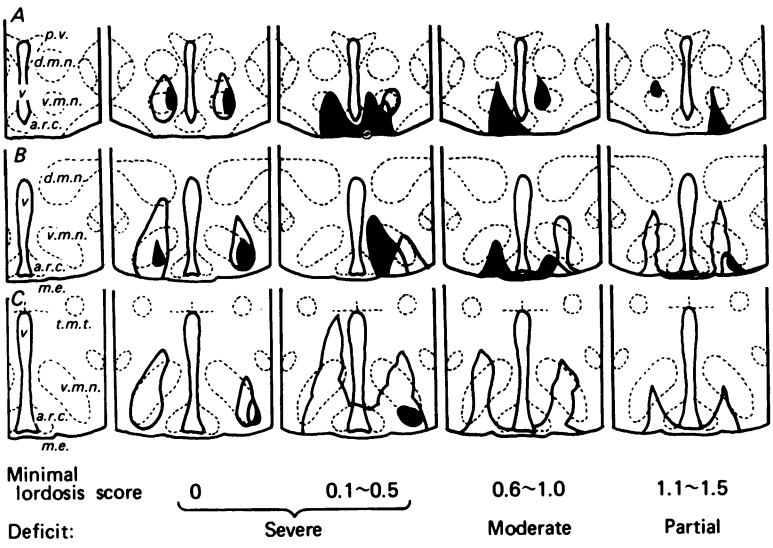

Fig. 4. Extent of lesions in the hypothalamus in animals with severe ($n = 11$), moderate ($n = 8$), and partial ($n = 9$) deficits in lordosis. Among animals with severe deficits, the lesions in animals which showed complete loss of lordosis ($n = 5$) are shown separately. Filled area, lesion in the rat with smallest damage in the ventromedial nucleus (v.m.n.). Open area, lesion in the rat with largest damage in v.m.n. The three sections are representative sections at 600 μm intervals. *A*, 2·0 mm caudal to bregma; *B*, 2·6 mm caudal to bregma; *C*, 3·2 mm caudal to bregma. Abbreviations: a.r.c., hypothalamic arcuate nucleus; d.m.n., dorsomedial nucleus; m.e., median eminence; p.v., paraventricular nucleus; t.m.t., mammillothalamic tract; v., third ventricle.

As may be seen in Fig. 4, the lesion tended to invade the lateral portion of the v.m.n. in animals with severe deficits of lordosis. A significant negative correlation was found between the magnitude of damage in the lateral half of v.m.n. and the post-lesion lordosis reflex score (Fig. 5). No such relationship was seen between loss in the medial half of v.m.n. and lordosis performance.

DISCUSSION

A gradual decline in the performance of lordosis followed v.m.n. lesions in oestrogen-treated ovariectomized female rats. The use of chronically implanted electrodes made it possible to follow changes without the interference of operative procedures. Since we used platinum-iridium electrodes, the possibility of irritative deposits can be excluded (see Donovan, 1966, for review).

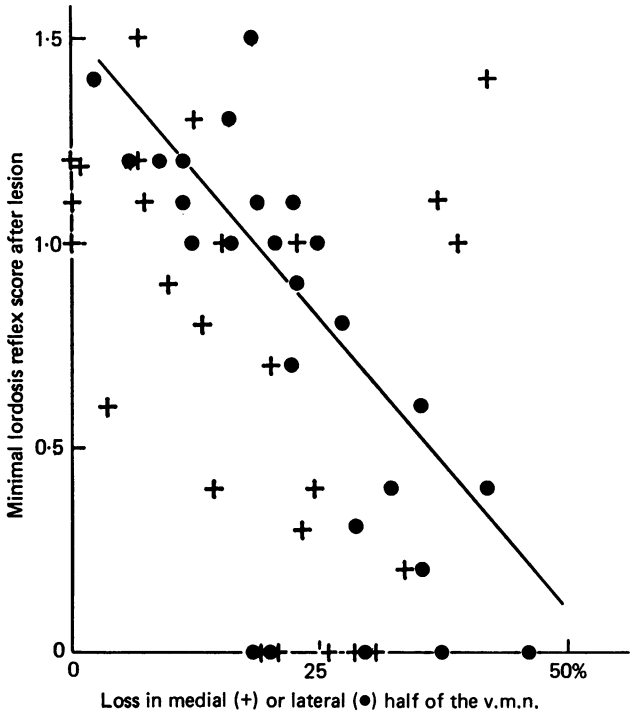

Fig. 5. Scatter diagram of the lowest lordosis score after the placement of the lesion in ventromedial nucleus (v.m.n.), *vs.* per cent loss of lateral (●) or medial (+) part of the v.m.n. in corresponding animals. Significant correlation ($r = -0{\cdot}79$; $P < 0{\cdot}01$) is seen between minimal lordosis score (S_{min}) and loss of lateral v.m.n. (V_L), but not between S_{min} and loss of medial part ($r = -0{\cdot}39$; $P > 0{\cdot}05$). Least-squares regression line predicts decrease in the lowest score as: $S_{min} = -0{\cdot}03\ V_L + 1{\cdot}51$.

Though the extent of the lesion varied from animal to animal, in no case was the greatest deficit of lordosis reached within 12 hr after v.m.n. lesion. This long latency probably is not due simply to gradual degenerative changes, because after identical lesioning procedures in the mesencephalic central grey lordosis loss was immediate (Sakuma & Pfaff, 1979). Therefore, the latency for the deficit probably reflects temporal properties of the mechanisms by which v.m.n. controls lordosis behaviour. For instance, a long latency was required for the facilitation of lordosis by electrical stimulation of v.m.n. (Pfaff & Sakuma, 1978). Moreover, neurones in the basal medial hypothalamus are known to have slow firing rates and to respond sluggishly, at best, to external stimuli (Bueno & Pfaff, 1976; Dyer, Pritchett & Cross, 1972; Lincoln, 1967). Thus, although present evidence does not provide an explanation

of the mechanism underlying the long time course, it can be concluded that the v.m.n. could not be in the direct reflex-arc for the lordosis response, which occurs 160 msec after stimulation (Pfaff & Lewis, 1974). In contrast, lesions of the midbrain central grey (Sakuma & Pfaff, 1979), or lateral vestibular nucleus or medullary reticulospinal neurones (Modianos & Pfaff, 1978), which are involved in this reflex-arc, result in immediate loss of lordosis. Presumably, hypothalamic neurones control lordosis by exerting a tonic hormone-dependent bias on these brain stem reflex circuits. The axonal projections from v.m.n. over which this occurs have been determined using neuroanatomical techniques. (Krieger, Conrad & Pfaff, 1979).

The degree of lordosis deficit following bilateral lesions was significantly correlated with the amount of the v.m.n. destroyed, especially in the lateral part of the nucleus. This provided quantitiative confirmation of the observation of Mathews & Edwards (1977). The importance of the lateral part of the v.m.n. in sustaining female copulatory behaviour was also referred to by Kennedy & Mitra (1963). Our analysis provided no evidence that nearby hypothalamic areas other than v.m.n. are directly involved. Since implants of minute amounts of oestrogen in the v.m.n. can induce sexual receptivity in ovariectomized female rats (Barfield & Chen, 1977; Dörner, Döcke & Moustafa, 1968), and cells in the lateral portion of the v.m.n. show preferential uptake of radioactive oestradiol (Pfaff & Keiner, 1973), it is possible that the deficits in lordosis result from destruction of oestrogen-sensitive neurones in v.m.n.

Increased reactivity to external stimuli has been observed by several investigators in rats with v.m.n. lesions (Kennedy, 1950; Marshall, 1975). In the present work the exaggerated responses could be induced from the anterior part of the body, in accordance with Marshall (1975), and not from the posterior part where appropriate stimuli elicit lordosis (Kow & Pfaff, 1976). However, it still is possible that an exaggeration in reactivity is related in some way to the deficits in lordosis.

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Y.S. is on leave from the University of Gunma Medical School, Maebashi, Japan.

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