Mesencephalic mechanisms for integration of female reproductive behavior in the rat

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Sakuma, Yasuo, and Donald W. Pfaff. Mesencephalic mechanisms for integration of female reproductive behavior in the rat. Am. J. Physiol. 237(5): R285-R290, 1979 or Am. J. Physiol.: Regulatory Integrative Comp. Physiol. 6(3): R285-R290, 1979.—Placement of bilateral electrolytic lesions in the mesencephalic central gray (CG) of estrogen-primed ovariectomized female rats produced an immediate decline in performance of the lordosis reflex. Lesions that destroyed the dorsal half of the CG and the adjacent subtectal region were effective. The decrease in individual animals in terms of the lordosis reflex score ranged from 20 to 100% of the prelesion performance. Such lesions abolished the facilitation of lordosis by electrical stimulation of the ventromedial nucleus of the hypothalamus. Similar abrupt losses of lordosis followed bilateral lesions of either a) the area between CG and the cuneiform nucleus of the mesencephalic reticular formation (NCF); or b) the ventrolateral quadrant of the NCF. The difference between these two lesions was that the effect of the latter could be overridden by electrical stimulation of the CG, whereas that of the former could not. We conclude that the CG is an important supraspinal component of the circuit for lordosis behavior, constituting a link between ascending somatosensory and descending motor systems for lordosis. It probably facilitates lordosis when activated by behaviorally relevant peripheral somatosensory and/or ventromedial hypothalamic inputs.

although electrical stimulation of the central gray (CG) of the mesencephalon has been shown to facilitate the lordosis reflex (27), mesencephalic substrates essential for normal reflex performance have not been defined. Possible ascending (16) and descending (11, 26) systems that could convey lordosis-relevant impulses (12, 18) to and from these regions have been described. The ventromedial hypothalamus is involved in the estrogenic induction of lordosis (1, 15), but is unlikely to carry this out in a reflexive manner to somatosensory stimuli (2, 23). Because supraspinal components play an essential role in the execution of lordosis (12), it seems likely that interactions between relevant ascending somatosensory and descending motor systems take place in the brain stem. Sensory convergence has been observed on neurons at various levels of the brain stem, from the pontomedullary reticular core (20, 32) to the mesencephalon (25). Among those, the roles of neurons in the mesencephalon are of a particular interest, since mesencephalic regions are where hypothalamic outputs crucial for lordosis intersect with relevant somatosensory inputs (3, 4, 13, 23, 24). The present experiments were designed to locate in the mesencephalon functional links between ascending and descending systems that govern the lordosis reflex.

Methods

A total of 54 albino female rats of Sprague-Dawley strain were used in these experiments. They were purchased from Hormone Assay Laboratories, Chicago, Illinois, and were ovariectomized at least 2 wk before the beginning of experimentation. During the course of behavioral observation, they were housed singly in a controlled environment at 24°C with a reversed 12-h light-dark cycle (lights off at 9 A.M.). Free access to laboratory chow and water was allowed at all times.

Lesion Experiment

Preparations. Two monopolar electrodes, Teflon-coated 10% iridium-platinum wire 178 μm thick, were chronically placed bilaterally in the CG of 28 animals. Procedures for the implantation of electrodes were described in the preceding paper (27), except that these animals had no indifferent electrodes. Daily injection of 2.5 μg estradiol benzoate (subcutaneously in oil) was begun on the day of the surgery and continued for about 20 days until high lordosis performance was obtained. Three animals with electrodes failed to show strong lordosis reflexes and were discarded at this stage.

Placement of CG lesion. Bilateral electrolytic lesions were made by passing DC anodal current of 1.0 mA for 15 s through each electrode. A rectal electrode was used as a cathode. For the observation of the time course of the change induced by the lesion, unanesthetized rats were put in a restrainer and the current passed. Lesions were made separately on each side. In no animal did application of the lesion current elicit seizure, unusual posture, or other complications.

Testing procedure. Lordosis was induced by manual cutaneous stimulation of the flanks followed by pressure on the rump-tailbase-perineum region (22). Quantitative determination of the lordosis performance in each animal was made by applying manual cutaneous stimuli five times, at brief intervals. Lordosis reflex strength on each stimulus application was rated using a scale from zero (no vertebral dorsiflexion) to three (strongest possible response) and the average of five ratings was calculated. This averaged value we termed the "lordosis reflex response" and central gray; reticular formation; estrogen.
score.” Collection of numerical data is discussed in the preceding paper (27). Prior to the placement of the lesion, the lordosis reflex score was confirmed to show stable values for at least 3 consecutive days. Following lesion, the lordosis reflex score was determined at intervals not greater than 2 h during the first 12-h period on the day of the lesion (day 0) and on day 1, and, subsequently, reflex tests were made once daily. Daily injection of 2.5 µg estradiol benzoate was continued throughout.

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

All reflex scores and lordosis quotients were converted by arc sine transformation, and compared by t test or analysis of variance.

Hypothalamic stimulation after CG lesion. For the examination of effects of CG lesions on hypothalaminically induced facilitation of lordosis, six animals were implanted with a pair of electrodes in the ventromedial nucleus of the hypothalamus (VMN), in addition to the CG. These animals received single injection of 5 µg estradiol benzoate on the day of the surgery, and monopolar stimulation of the VMN was given on the 4th postoperative day. A jeweler’s screw on the frontal bone was used as indifferent electrode. Parameters for the stimulation were: frequency, 10 Hz; intensity, 25 µA; pulse duration, 0.2 ms; and the separation of square-wave pulses within each balanced biphasic pair, 0.1 ms. The train of pulses was continued for 30 s and interrupted for another 30 s, repeated alternately for 2-5 h. Lordosis was induced manually and rated using the lordosis reflex score. In the four of six animals in which VMN stimulation successfully facilitated lordosis, bilateral electrolytic lesions were placed in the CG. After an interval of 2 wk from the initial injection of estrogen a second dose of 5 µg estradiol benzoate was given 2 wk after the first injection. Four days after the injection, these animals were subjected to bilateral electrolytic lesions in the locations referred to in Table 1. Lesions were produced in the same manner as described earlier for the CG. After another interval of 2 wk, CG stimulation was used again with these lesioned animals, under comparable treatment with estrogen. Facilitative effects of the CG stimulation, if any, were compared in each animal, between the data collected before and after and of the lesion.

Histological Analysis

In both the lesion and combined lesion-stimulation experiments, animals were anesthetized with an overdose of pentobarbital sodium (Nembutal) at the end of observation, and transcardially perfused with 10% formalin. The site and extent of the lesion, as well as the placement of electrodes in the lesion-stimulation experiment, were reconstructed in frozen sections 50 µm thick, stained with luxol fast blue and cresyl violet.

RESULTS

Lesion Experiments

Deficits in lordosis. In 25 ovariectomized female rats with electrodes implanted chronically in the CG, daily estrogen treatment effectively sustained strong and rapid lordosis. Bilateral electrolytic lesions through these electrodes resulted in an immediate and significant decrease in the lordosis performance, both in terms of the lordosis reflex score to manual cutaneous stimuli ($P < 0.01$, t test) and the lordosis quotient to male mounts ($P < 0.05$) (Fig. 1). The deficit appeared immediately, as illustrated in Fig. 2. The decrease in individual animals ranged between 20 and 100% of prelesion control score. Complete (100%) loss of lordosis, restricted to four animals tested 2 min after lesion, did not persist. As can be seen in Fig. 2, some recovery in the lordosis score usually followed the initial decrease, and lordosis performance settled at low values for up to 3 wk without a significant further recovery, under continuous treatment with estrogen.

Associated changes in sensorimotor activity. These lesions produced no gross disorders in motor functions. We noticed, however, that in many animals locomotion was clumsy immediately after the lesion, that there appeared to be decreased tonus of the axial and hindlimb musculature, and extensive licking activity of the trunk

| TABLE 1. Coordinates for implantation sites other than central gray |
|-----------------|-------|-------|
| A-P | Lateral | Depth |
| Rostralmost part of NCf | 2.2 | 1.7 | 5.0 |
| Lateral part of NCf | 0.2 | 1.9 | 3.7 |
| Area between CG and NCf | 0.2 | 1.2 | 4.0 |

NCf, cuneiform nucleus; CG, central gray. Details of stereotaxic procedure are given in METHODS.
SEXUAL IMPAIRMENT FROM MESENCEPHALIC LESIONS

These activities. Although no systematic analyses were made of these latter responses, which resembled copulatory behavior of male rats, it was clear that these CG lesions disrupted only the female-typical pattern of reproductive behavior.

**Histological results.** In many animals the CG lesion extended dorsally into the area deep to the superior colliculus. In the rostrocaudal direction, all lesions were centered approximately at the midsuperior collicular level. None extended beyond the lateral or ventral borders of the CG. Oculomotor nucleus, trochlear nucleus, and the nucleus of the mesencephalic root of the trigeminal nerve were always intact.

To determine the loci in which damage produced the largest deficits in lordosis, 25 animals were divided into three groups according to the magnitude of lordosis deficit, and composite histological sketches were prepared for each group (Fig. 3). The largest deficits followed bilateral damage in the dorsal half of CG, which extended into the area deep to the superior colliculus.

Effects of CG lesions on hypothalamically induced facilitation of lordosis. The lordosis reflex can be aug-

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**FIG. 1.** Effects of central gray lesions on lordosis reflex score (shaded bars) and lordosis quotient (open bars) in estrogen-primed ovariectomized female rats. n, Number of rats; Pre, prelesion lordosis performance. Vertical bars denote SEM. Significant decreases in score (*P < 0.01, analysis of variance) and lordosis quotient (**P < 0.05) followed lesion. There was no statistically significant recovery after 20 days.

**FIG. 2.** Time course of changes in lordosis reflex score following central gray lesions in 3 representative animals. Lesions were made bilaterally at 0 min (arrow). Daily subcutaneous injections of 5 μg estradiol benzoate were given throughout period shown.

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was seen. These abnormal behaviors, which persisted for 4–5 h after lesion, gradually disappeared. Corneal, grasp, righting, and withdrawal reflexes were normal. When placed with other sexually receptive female animals, lesioned animals showed vigorous and repetitive mounting and thrusting, and licking of the genital area followed...
mented in estrogen-treated ovariectomized female rats by electrical stimulation of the VMN (23). As the CG is known to have reciprocal connections with the hypothalamus (3, 4, 10, 13, 19, 26), the possibility that the CG might be involved in mediating the effect of VMN stimulation was investigated.

Bilateral CG lesions effectively abolished VMN-induced facilitation of lordosis. Before placement of CG lesions, rats were capable of responding during VMN stimulation with the strongest possible lordosis reflex. As shown in Fig. 4, VMN stimulation, though given for a longer period than prelesion control, failed to increase lordosis when repeated under identical estrogen treatment 2 wk after CG lesion.

**Studies with CG Electrical Stimulation and Mesencephalic Lesions**

To study the manner in which CG affects lordosis behavior, we lesioned possible ascending and descending conduction systems for CG (8-10, 14, 19, 26), and observed the effects of these lesions on lordosis and on the lordotic reflex facilitation during electrical stimulation of CG (shown in the preceding report to be effective in facilitating lordosis, Ref. 27).

**Sites of mesencephalic lesions.** Mesencephalic lesions were placed at three different rostrocaudal levels. At the level of the posterior commissure, bilateral lesions were made in the rostralmost part of the cuneiform nucleus of the mesencephalic reticular formation (NCF). At the intercollicular level, lesions invaded areas immediately lateral to the CG. Caudally, attempts were made to destroy the area between CG and NCF, at the caudalmost level of the latter structure. In a fourth group of animals, lesions were placed in the ventrolateral quadrant of NCF at the same caudal level.

**Effects of lesions on lordosis.** Two placements of bilateral lesions, both situated at the caudalmost level of NCF markedly lowered lordosis performance: in the cell-poor area between CG and NCF and in the ventrolateral quadrant of NCF. Following the former lesion, mean lordosis reflex score declined from 1.4 to 0.1 (mean percent decrease 93%) in three rats. This lesion infringed on part of the ventral CG and the trochlear nucleus or the nucleus of the mesencephalic root of the trigeminal nerve, but resided chiefly in the cell-poor area ventrolateral to the CG (Fig. 5). The other type of lesion caused a decrease of 69% in the score, from 1.5 to 0.5 in the mean of eight animals. This lesion was located in the ventrolateral quadrant of the NCF, sometimes extended more laterally to the area between NCF and the lateral lemniscus, but medially the lesion always remained within the border of the NCF. Other lesions, including the rostralmost part of NCF, had little effect on lordosis (mean percent decrease 16%). Examples of large ineffective lesions are shown in Fig. 6 to prove absence of nonspecific effects of the brain stem lesion on the lordosis reflex.

![Fig. 5. Composite diagram of lesions in mesencephalic reticular formation that effectively disrupted lordosis. Cross-hatched lesions in lateral central gray (CG) and area between CG and cuneiform nucleus (NCF) abolished facilitative effect of CG stimulation. Loss of lordosis also followed lesion of lateral NCF (open areas), but CG stimulation effectively counteracted this type of lesion effect. Numbers in upper left corner of diagrams indicate rostrocaudal distances in mm from bregma. LL, lateral lemniscus; MR, median raphe nucleus; for other abbreviations see Fig. 3.](#)
CG stimulation in lesioned animals. Following NCF lesions at the level of posterior commissure, CG stimulation facilitated lordosis performance as well as in the prelesion sessions (Fig. 7A). Similarly, in animals with lesions in the ventrolateral quadrant of the NCF at its caudalmost level, the mean percent increase in the lordosis reflex score induced by CG stimulation was 67%, which was as large as the prelesion value (Fig. 7B); because these animals had almost no ability to show lordosis without CG stimulation, the stimulation essentially counteracted the lesion effect.

In contrast, as shown in Fig. 7C, in animals with lesions between CG and NCF, CG stimulation had no facilitative effect on lordosis behavior.

**DISCUSSION**

An immediate decline in the performance of the lordosis reflex followed lesions of the CG in estrogen-primed ovariectomized female rats. The rapid effect of the CG lesion on lordosis contrasts with the more gradual decrease that followed lesions in the ventromedial nucleus of the hypothalamus (24). Whereas the VMN effect was considered (24) to result from loss of a tonic facilitatory influence on the reflex loop for lordosis, the immediate behavioral effect of the CG lesion is consistent with the notion that the CG is part of a reflex loop completed at a mesencephalic level.

The most effective lesions were in the dorsal half of the CG and extended into the subtectal area. Those lesions confined to the ventral part of the CG were less effective. Several anatomic and physiological studies refer to significant differences between dorsal and ventral subdivisions of the CG. In contrast to the pronounced fiber bundle descending from dorsal CG, there are only fine fibers traveling diffusely from ventral CG (26). Hamilton (8, 9) defined three subdivisions of the CG, based on shape and size of neurons, and reported that each has a different field of projection. Estradiol-concentrating cells in the mesencephalon have been demonstrated in the CG, with a few also in the subtectal area (21). With electrical stimulation, analgesia could be induced only from the ventral part of the CG (17); this topographical difference may imply a functional separation between CG-induced lordosis and analgesia.

Behavioral observations of decerebrate preparations indicate that a high degree of integration of various postural and locomotor responses can be called out by mesencephalic mechanisms (see Grillner, Ref. 7, for review). Shik et al. (reviewed by Shik and Orlovski, Ref. 28) have demonstrated induction of coordinated stepping in decerebrated cats by electrical stimulation of circumscripted structure. Their concept of "locomotor region" in the mesencephalon and pons may have some relevance to the present results, despite differences in the effective loci. Interesting coincidences exist in the experimentally induced stepping and lordosis. For instance, both require appropriate somatosensory stimuli in addition to electrical stimulation, and the effects of electrical stimulation in both cases are presumed to be mediated by the reticulospinal system to release activities of lumbosacral spinal neurons. In the present study interruption of projections from the anterolateral system of the spinal cord at the level of the posterior commissure had no significant effect on lordosis, indicating that somatosensory inputs to the diencephalon are probably not required for induction of lordosis. Thus, the impact of ascending information on descending systems for lordosis probably is completed at levels no higher than the mesencephalon.
Lesions of two additional sites in the mesencephalon outside the CG, namely the area between CG and NCf, and the ventrolateral quadrant of the NCf, are as effective as CG lesions for disrupting lordosis. CG electrical stimulation counteracted the deficit caused by lesions in the ventrolateral quadrant of NCf, but not the area between CG and NCf. Available anatomic data provide an explanation for these results. The lateral part of NCf contains the ascending projection of the anterolateral system of the spinal cord (14, 16, 19). Lesions here could interrupt transmission of relevant somatosensory input, thus diminishing the lordosis reflex. Our observation of successful induction of lordosis in these animals, using CG stimulation, further indicates that the efferent pathway of the CG was not disturbed in these animals. In turn, a well-defined fiber bundle leaves from the dorsolateral part of the CG (where electrical stimulation facilitates lordosis, Ref. 27) and travels into the NCf (10, 26). The effective lesion in the area between CG and NCf approximates the trajectory of this bundle. Thus, the loss of the CG-stimulated facilitation of lordosis following this type of lesion probably indicates severance of efferents descending from the CG. Some of these fibers terminate in reticulospinal nuclei in the medulla (26), which are importantly involved in the induction of lordosis (18).

The NCf itself is a major nucleus in the mesencephalic reticular formation of the rat (30). Descending projections of NCf terminate in the reticulospinal nuclei (5, 6, 31). In the cat, electrical stimulation elicited an asymmetrical posture followed by vigorous attack (29). In the rat, electrical stimulation of this structure produced contralateral contraction of axial musculature (27). Considering that unilateral stimulation was effective to promote lordosis from CG but not NCf (27), it might be concluded that in the reflex loop for lordosis, integration in the CG is indispensable for producing a coordinated bilaterally symmetrical response.

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