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SENSORY AND HYPOTHALAMIC CONTROL OF LORDOSIS BEHAVIOR IN FEMALE RODENTS

A thesis submitted to the Faculty of the Rockefeller University

in partial fulfillment of the requirements

for the degree of Doctor of Philosophy

by

Kirk R. Manogue

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The Rockefeller University

New York

PREFACE

I thank both of my advisors for their contributions to this effort. Dr. Donald Pfaff has not only provided essential guidance throughout my student career, but also gathered together a generously supportive research group, every member of which I thank for help along the way. Dr. Lee-Ming Kow's support and guidance have been most important to me and my work.

I would also like to thank Dennis Chattman, who provided a great deal of technical help in preparing my histology; and Birgit Rogers for her cheerful aid in preparing the manuscript.

ABSTRACT

When applied during the sustained lordosis response of female hamsters, gentle probing at points on the perineal surface elicited stereotyped rump and tail movements. The rump movements always served to rapidly center the vaginal opening beneath the continuing stimulation. Tail deflections were only elicited by stimulation applied above and lateral to the vaginal opening. The tail was deflected away from the side stimulated. These observations help to complete the description of the stimulus-response relations of the component reflexes which comprise the complete lordosis response in hamsters. The movements would serve to facilitate vaginal access during copulation and foster accurate targeting of penile thrusts to the vaginal opening.

Puffs of air directed to the lateral flank surface triggered lordosis in some estrogen-progesterone treated, ovariectomized female hamsters. This stimulation covered only a small area of the flank, and exerted very little pressure on deep structures, indicating that the deflection of a few hairs within a small area of the flank is sufficient to trigger lordosis in some hamsters. Prior exposure to the ministrations of a sexually active male hamster facilitated the triggering of lordosis by unilaterally applied air puffs, and potentiated the intensity of the lordoses exhibited. The expression of this effect did not require the activation of rump displacement reflexes by perineal stimulation during the post-male test. Light brushing of flank hairs, on one or both flanks elicited

lordosis as reliably as post-male, unilateral air puffs. Increasing the area or intensity of artificially applied stimulation triggered more intense lordosis responses. These observations help to define the minimal sensory stimulus required to trigger lordosis in hamsters, providing information useful in the neurophysiological analysis of this hormone dependent reflex.

In estrogen-replaced,ovariectomized rats, selective transections were used to interrupt, together or separately, the medial and lateral pathways by which efferent fibers from the ventromedial nucleus of the hypothalamus reach the lower brainstem. Transections interrupting both projections reduced or eliminated lordosis performance. Transections which intercepted all of the medially descending fibers, but spared the lateral pathway, did not reduce lordosis performance in mating or manual stimulation tests. The lateral pathway was interrupted at two different locations. The lateral pathway, as a whole, was not required for lordosis when the medial pathway was left intact. Also, no particular subset of fibers assuming a lateral trajectory from the VMN to the brainstem were required for the display of lordosis. However, the fibers running through the lateral brainstem do play some role in the expression of the reflex - their transection bilaterally did reduce lordosis performance. The failure of lordosis to occur in mating tests was not a result of a systematic increase in rejection behavior. The observation of intermittent lordosis responses, or increased lordosis performance following additional estrogen and progesterone treatment,

revealed that these transected animals were still able to produce the motor behavior required for lordosis. The deficits seen were attributed to the interruption of fibers mediating the control of lordosis by the hypothalamus. This control of lordosis by the ventromedial nucleus can be described as a tonic, estrogen-dependent facilitation of supraspinal lordosis control mechanisms located more caudally in the brainstem. The laterally, rather than the medially, descending efferent fibers from the ventromedial nucleus may play a quantitatively more important role in the control of lordosis.

GENERAL INTRODUCTION

During copulation, female rodents adopt a stereotyped posture - lordosis - which presents the vaginal opening in a favorable position for intromission to occur. Broadly speaking, two factors interact in the elicitation of lordosis. Internally, the neural control mechanism for lordosis must have been correctly conditioned by the ovarian hormones - estrogen and progesterone. Externally, appropriate sensory stimulation must be delivered onto specific portions of the body surface. The distinction between a sensory mechanism which triggers the lordosis reflex, and a hypothalamic mechanism through which hormonal influences are exerted, has guided my selection of the more suitable preparation in which to study the control of lordosis.

Hamsters are ideal subjects for the study of the externally applied stimulation relevant to lordosis. Female hamsters lordose reliably to both natural, male applied stimulation; and to artificially applied stimulation which mimics copulatory stimuli. Furthermore, hamster lordosis is sustained over many seconds, allowing a good opportunity to closely assess the involvement of component reflexes which make up the complete lordosis response. Therefore hamsters were used in the experiments reported in Chapters 1 and 2. These experiments describe some of the stimulus-response relations which are observed with lordosis-relevant perineal (Chapter 1) and extra-perineal (Chapter 2) stimulation.

The experimental manipulation of central nervous tissue to determine the mechanism by which internally derived hormonal influences control the expression of lordosis is more conveniently studied in female rats. Rats are slightly larger, hardier following surgical trauma, and more of the necessary neuroanatomical background information is available. Therefore rats where chosen as the preparation of choice in which to determine the course of hypothalamic fibers which mediate the estrogen-dependent control of lordosis.

CHAPTER 1

SPECIFICITY OF RESPONSES TO PERINEAL STIMULATION IN FEMALE HAMSTERS

INTRODUCTION

The identification of neural circuits controlling mating behavior in rodents has been greatly aided by the precise description of the orderly stimulus-response relationships which occur throughout the elicitation of the lordosis reflex in female rats. Frame-by-frame analysis of film (Pfaff and Lewis, 1974) and X-ray (Pfaff, et al., 1978) cinematographic records of mating rats has provided a precise description of the areas stimulated by the male during a copulatory mount, and of the movements made by females in assuming the lordosis posture in response to this stimulation. The experimental manipulation of stimulation applied by the male (Kow and Pfaff, 1976), and the analysis of the female's responses to artificially applied stimulation mimicing different aspects of the male-applied stimuli (Pfaff, et al., 1977) have generated a formal description of the stimulation sufficient and necessary to elicit components of the lordosis reflex. The synthesis of these detailed observations has generated a "cascade" or "sequential reflex" model of lordosis by female rats (Pfaff, et al., 1973). According to this model, the solicitation behavior of the female rat tends to align her body axis so that the first contacts by the male are delivered (from the rear) to the female's flanks and back. The initial responses by the female (leg extension, rump and tailbase elevation) and postural adjustments by the male allow more intense stimulation of the female's rump, groin and tailbase. The "cascade effect" of these initial interactions

exposes the female's perineal area to thrusting stimulation by the male. The combined stimulation on all of these skin zones, including the perineum, is required for the full expression of the lordosis reflex in female rats. The full motor pattern of lordosis is achieved, however, without intravaginal stimulation.

Sexual interactions between a male and a female hamster typically begin with a period of exploration of the female's perineum and vaginal opening by the male. The male noses, sniffs, and licks the female's perineum, apparently attracted to olfactory components of the female's vaginal discharge (Powers, Fields, and Winans, 1979). This perineal stimulation is frequently accompanied by stimulation of the female's lateral flank by the paw of the male as he steadies himself to twist his head and lick the perivaginal area. Receptive females generally respond to these initial contacts by displaying the lordosis posture (Chapter 2 for description). The resultant exposure of the perineum in a nearly vertical plane presents a flattened surface on which the nosing and licking by the male takes place. As the male licks across this surface, postural adjustments and tail movements by the female are apparent. The female does not break from the lordosis posture, but her rump and tail are seen to twitch from side to side. The female's rear feet are not lifted from the substrate, nor moved to locations different from those occupied during assumption of the lordosis posture. These rump and tail movements are seen during perineal exploration by the male before the first mount and during other

periods of like stimulation which may be interspersed between successive mounts in the mating sequence.

The close apposition of the male's head to the female's perineum makes these motor adjustments, and the stimuli eliciting them, difficult to observe as they occur. In order to provide a brief description of these stimulus-response relations, cutaneous stimulation mimicing that supplied by the male can be applied near the vaginal openings of female hamsters. Furthermore, the influence of gonadal hormones on this stimulus-response relationship can be conveniently studied in hormone-treated or untreated, ovariectomized female hamsters.

METHODS

Postural and tail position adjustments in response to perineal stimulation were studied in the same group of female hamsters described in Chapter 2. Observations were made at two different times during the fifth injection and testing cycle of the flank stimulation experiments (see Methods, Chapter 2). Perineal stimulation was applied to each female just prior to the EB injection of the fifth cycle, and after the completion of the flank stimulation testing regimen of that same cycle. Thus, responses to perineal stimulation were assessed in ovariectomized, untreated (7 days post-EB, 5 days post-P) and ovariectomized, hormone-treated (46-50 hours post-EB, 5-9 hours post-P) female hamsters. Many of these females were stimulated in a variety of ways on other EB+P

injection cycles in order to develop a description of the tail deviation and lordosis posture adjustment reflexes. Effectiveness of hormone replacement, with respect to lordosis responding, had already been established (see Chapter 2).

Perineal stimuli were applied with the rounded wooden end (3 mm. diameter) of a small paint brush and consisted of repeated 1 cm. strokes at each of four locations (Figure 1-1). The strokes were oriented vertically and were delivered in each of the four quadrants of a Cartesian plane one can imagine superimposed on the perineal surface (see Fig.1-1). The origin of the Cartesian coordinate system corresponds with the center of the vaginal opening after a lordosis response has been elicited. The middle of each stimulation stroke was located approximately one cm. above, or below, and the right, or left of the Cartesian axes. As postural adjustments occurred, the site of stimulation was maintained at the same topographical location on the perineal surface. Perineal probing during other EB + P injection cycles was not so restricted. A variety of stimulation techniques and locations were employed in developing a description of the reflexes.

In order to elevate the rump and tail to expose the perineum for application of stimuli, each female was manually palpated. A test female, standing on a flat surface, was grasped across the back and around the sides with the middle, ring, and little fingers of the experimenter's left hand. While squeezing the flanks of

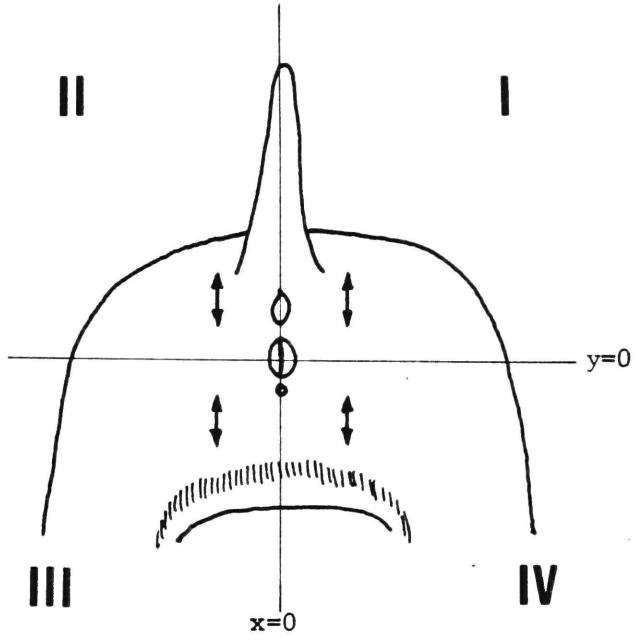

Figure 1-1. Reference Cartesian axes superimposed on a schematic rear view of a lordosing female hamster. Origin (0,0) centered on vaginal opening. Arrows indicate location and direction of applied stimuli in quadrants I, II, III, and IV.

the female's rear legs between his fore-finger and thumb, the experimenter pulls caudo-rostad along the females flank. Any necessary upward force to this palpation can be added to expose the perineal surface in a nearly vertical plane. This palpation holds non-receptive females in a lordosis-like configuration, and is sufficient to trigger strong lordoses in receptive females (see Chapter 2). Palpation during the test applications of perineal stroking was continued as necessary to maintain access to the perineal surface.

RESULTS

General description of stimulus-response relationships.

Tail deviations and postural adjustments are most easily observed and described when elicited as changes in the lordosis response of the receptive female. Three regularities are immediately evident in the stimulus-response relationships of these adjustments to the lordosis posture (Table 1-1). First, stimuli applied on the right or left side of the vaginal opening elicit a lateral displacement of the rump towards the side of applied stimulation. Second, the entire rump is elevated in response to stimuli applied above the vaginal opening and lowered in response to stimuli applied below the vaginal opening. Third, stimuli applied above, but not below , a horizontal line bisecting the vaginal opening elicit tail deflections away from the side of stimulation.

This bilateral symmetry and dorsal-ventral assymmetry suggest a convenient frame of reference for discussing the topography of

Quadrant stimulated	Tail deviates to:		Rump displaced:			
	Right	Left	Up	Down	Right	Left
I	0	14	14	0	14	0
II	14	0	14	0	0	14
III	0	0	0	14	0	14
IV	0	0	0	14	14	0

Table 1-1. Direction of tail deviations and rump displacements in response to stimulation at each of the perineal quadrants. Fourteen ovariectomized, estrogen-progesterone treated female hamsters were stimulated once within each quadrant. Numbers in the table indicate the number of responses in that direction.

stimulus and response. This reference can be pictured as a right Cartesian plane whose origin is centered on the vaginal opening when the full lordosis posture is elicited (Figure 1-1). Postural and tail position adjustments will be reported as observed in response to cutaneous stimuli applied to the first and fourth quadrants of this perineal plane. Bilaterally symmetric responses were observed in the second and third quadrants, respectively.

Perineal stimulation in the first quadrant consistently elicited the same sort of postural adjustments and tail deviations. These were expressed within the framework of the more global lordosis reflex. When light cutaneous or pressure stimulation was applied to the perineum, the entire hindquarters of the lordosing female shifted dorsally and laterally directly toward the specific site of stimulation. The most prominent (visually apparent) motor correlate of this rump displacement was extension of the left (contralateral) leg, especially around the knee joint. When the site of stimulation was held fixed with respect to the original Cartesian origin, movement of the rump stopped when the vaginal opening centered under the source of continuing stimulation. When the source of stimulation was moved to maintain the same topographical separation from the vagina, movement stopped when extension of the contralateral leg was maximal (see Fig. 1-2). The feet were never moved to continue tracking the stimulus. Rump movements were accurately targeted to the site of stimulation. Graded responses were seen, appropriate to bring the vaginal opening to different locations of stimulation

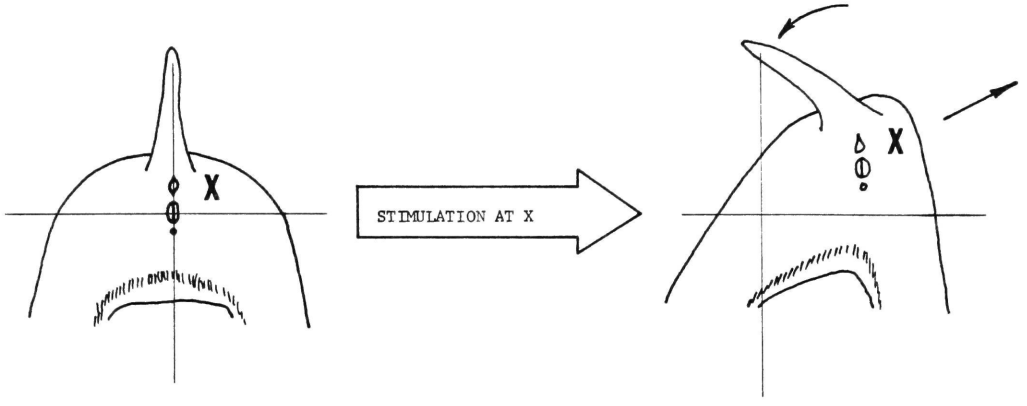

Figure 1-2. Schematic rear view of lordosing female hamster before and during stimulation at point X. Arrows indicate direction of tail deflection and rump displacement.

within the first quadrant. Stimulation along the midline (x=0) elicited a bilaterally symmetric extension of the rear legs and elevation of the rump.

Tail deflections occurred in the opposite direction as rump displacements. The tip of the tail (which is held straight up at strong lordosis intensities) was moved promptly away from the side of applied perineal stimulation in the first or second quadrant (see Fig. 1-2). When the site of stimulation was held fixed in the Cartesian plane, tail deflections returned to zero degrees (from vertical) as the vagina was rapidly centered under the site of continuing stimulation. If the site of stimulation was maintained at some remove from the vaginal opening, tail deviation was also maintained. Stimuli along the midline did not elicit lateral tail deflections.

When a series of stimuli were delivered to the perineum and adjusted to maintain their original separation from the vaginal opening, rump displacement could only proceed until further extension of the contralateral leg was impossible. When stimulation was then stopped, the rump displacement and tail deflection persisted until lordosis terminated, or perineal stimuli were applied at a different location.

Stimuli applied to the fourth perineal quadrant also elicited rump displacements toward the side stimulated. This movement involved

a ventrad, laterally directed twisting of the hindquarters bringing the vaginal opening to the location of the applied stimulus. Stimulation along the midline did not elicit lateral twisting, the rump was symmetrically lowered. As the rump was lowered, the perineum was maintained in a nearly vertical plane, parallel to one approximated by the initial assumption of the lordosis posture.

Stimuli in the fourth (and third) quadrants did not elicit tail deflections.

Responses by hormone-treated v. untreated ovariectomized females.

Hormone-treated (EB + P) females showed stereotyped rump and tail postion adjustments in response to stroking stimuli of restricted areas of the perineum. The tail deflections and rump displacements corresponded to those described above, quadrant for quadrant. No other responses to these stimulations were observed apart from an intensification of the sustained background lordosis reflex on which the adjustments are superimposed. The intensity of lordosis reached EXTREME (see Chapter 2) in all females when perineal stimulation was added to a constant, gentle flank palpation. Perineal stimulation was sufficient to maintain STRONG or MODERATE lordoses indefinitely. Thus flank palpation could be terminated for observation of tail and rump position adjustments. Threshold for eliciting the adjustments (and for maintaining lordosis) was low. Light tapping at a point on the perineum was sufficient; vigorous stroking effective, though such high pressure levels were not necessary. All rump and tail

	UNTREATED		EB + P REPLACED	
FEMALE NUMBER	LORDOSIS INTENSITY[1]	TAIL & RUMP REFLEX INT.	LORDOSIS INTENSITY	TAIL & RUMP REFLEX INTENSITY
11	moderate	strong	extreme	strong
12	poor	weak	extreme	strong
13	poor	weak	extreme	strong
14	poor	weak	extreme	strong
16	absent	weak[2]	extreme	strong
17	absent	absent	extreme	strong
18	poor	weak	extreme	strong
19	strong	strong	extreme	strong
20	poor	weak	extreme	strong
23	strong	strong	extreme	strong
24	strong	strong	extreme	strong
25	moderate	strong	extreme	strong
28	moderate	strong	extreme	strong
30	moderate	strong	extreme	strong

1. Lordosis intensity was assessed during combined manual palpation and perineal stroking, and indicates the maximum achieved during that period.

2. weak in 1st quadrant, absent in the other three.

Table 1-2. Strength of lordosis reflex, and tail and rump reflexes elicited in hormone-treated, and untreated ovariectomized female hamsters. Each female was tested for tail and rump responses in each of the four perineal quadrants.

position adjustments to stimuli applied to the four quadrants were consistent with the general description above. All of these qualities contributed to assessment of the tail deviation and rump displacement reflexes as STRONG in hormone-treated females (see Table 1-2).

Rump and tail position adjustments were also observed in response to perineal stimulation in "untreated" ovariectomized females. However, these movements did not always conform to the descriptions above. A number of potentially competing motor adjustments were observed on an inconsistent background of partial lordosis reflexes.

Lordosis responses were not generally elicited in these females by vigorous manual palpation alone -- locomotion frequently continued as the animal struggled to escape the experimenter's grasp. As vigorous strokes were applied to the perineum, locomotion usually ceased,and some intensity of lordosis could be credited (12/14, see Table 1-2). These "lordoses responses" generally achieved only low intensity, were in part due to direct physical control of the attitude of the hindquarters by manual palpation to expose the perineum, and were not always maintained even with continuous flank palpation and perineal stroking. Participation of the rostral half of the hamster in these "lordosis responses" was especially poor, or absent.

Frequently (6/14), rump position adjustments did not appear as promptly initiated nor as vigorous as those observed in EB + P treated females. Furthermore, rump position and tail deflection movements not consistent with those described above were seen in response to perineal stroking (e.g. differing in direction). These factors con-

tributed to the assessment of the tail deflection and rump displacement reflexes as WEAK or ABSENT in these females (see Table 1-2). A more precise appraisal of the strength and integrity of the response is difficult as it must be assessed during a firm grasp and vigorous manual palpation of the female. With untreated females, very firm pressure was applied on the perineal strokes. In some females this high pressure elicited postural adjustments consistent with the previous description at a higher probability than other movements. These females showed the same pattern of reflexive rump and tail movements described for hormone treated females, i.e. postural adjustments bringing the vaginal opening towards the site of stimulation, and deflecting the tail away from the side stimulated. These patterned responses predominated over other movements (struggling) and were scored as STRONG (Table 1-2).

DISCUSSION

Rump and tail displacements occur reliably and with stereotyped topography in response to cutaneous stimulation of small patches of the perineal skin of sexually receptive female hamsters. These adjustments are always seen superimposed on the more global lordosis reflex displayed by hormone replaced(EB + P) ovariectomized females. When the same females were tested long after hormone replacement, the reliability of elicitation of these stereotyped responses was greatly decreased. Those females reliably showing these responses in the "untreated" condition also showed the more intense lordosis responses to the synergistic stimulation of combined flank and perineal stimulation. Females from which lordosis could not be elicited, or from which minimal lordosis intensities were observed, showed weaker tail and rump displacement reflexes and many more competing movements (struggling, rump movements away from the site of stimulation, locomotion).

In rats, perineal stimulation is required for the full expression of lordosis; manual stimulation which does not include the perineum very rarely elicits the full, intense lordosis response (Pfaff, et al., 1973). Furthermore, the symmetry of the response of the female is not affected by the side of the tail (right or left) from which the male angles his thrusts to the vaginal opening (Pfaff and Lewis, 1974). In other words there is not a systematic lateral movement of the female's rump towards or away from the side from which

the male thrusts. The female's "cascaded" series of responses during lordosis presents the vaginal opening in a favorable position for intromission to occur, but the fine targeting for penile insertion is accomplished by the male. In hamsters, the full lordosis response can be triggered by stimulation of the flanks or dorsal rump alone (see Chapter 2; Pfaff, et al., 1973; Kow, et al., 1976). Perineal stimulation is not required, and female hamsters can show intense lordosis before the male mounts. In this sense, hamsters do not show the obligatory "cascade" of responses to initial stimuli which expose perineal areas to the stimulation required for the full lordosis response of female rats. In hamsters, perineal stimulation does intensify sub-maximal lordoses, but it also plays an additional role -- targeting the vaginal opening to the source of stimulation (rump displacement) and facilitating access to the vaginal opening (tail deflection). While perineal stimulation serves only to present the vaginal opening favorably for intromission in rats, such stimulation enables the female to contribute to the fine targeting of penile thrusts to the vagina in hamsters.

In rats, most of the perineum is innervated solely by the pudendal nerve (Kow and Pfaff, 1973). A similar exclusive innervation in hamsters would implicate the pudendal nerve as the afferent limb of these specialized perineal reflexes. These pudendal influences must be integrated differently from other lordosis-adequate sensory inputs from the hamster rump and flanks which trigger lordosis movements. Unilateral flank and rump stimulation does not elicit laterally assymetric lordosis responses in hamsters (see chapter 2).

CHAPTER 2

ANALYSIS OF ADEQUATE STIMULI FOR ELICITING LORDOSIS IN FEMALE HAMSTERS

INTRODUCTION

In most laboratory testing situations where retreat from a social encounter is impossible for either partner, encounters between an adult male and an adult female hamster eventuate one of two behavioral sequences. The two alternatives, one aggressive, one sexual, are correlated with the typically four-day estrus cycle of the female hamster. Three days out of four, interactions between the partners culminate in overt, vicious fighting. On the day of estrus, however, such encounters lead to the mutual, reactive display of copulatory behaviors. The receptive female's sexual behavior repertoire is characterized by adoption and maintenance of a sustained, immobile postural configuration, the lordosis reflex. The topographical extent of this response by the female is global. The back of the female is held concave down, with rigid extension of the rear legs and elevation of the rump exposing the perineal surface. The tail is held up. The body is rocked forward over the front feet and the stance is held on the toes of the rear feet. The head and facial musculature are still, vibrissae movement stopped, eyes staring fixedly ahead, ears still. The underlying musculature of the back and dorsal sides appears rigidly contracted and clearly defined beneath skin pulled tight by the extension dominated posture. This motor complex can be sustained for tens of minutes in response to appropriate stimulation by the male partner.

Observation of mating hamsters indicates that appropriate

stimulation can be supplied by the male during the most initial phases of the encounter. The complete motor complex of the female's lordosis reflex is frequently elicited by the male nosing and sniffing her lateral flank, or placing a paw on one flank and licking her perineal area. These behaviors by the male may persist for several minutes before the clasping, mounting, thrusting, and intromissive behaviors of the full mating sequence begin. The female is generally seen to adopt and maintain the lordosis posture in response to these initial contacts, whatever their duration, before any of the more vigorous (and bilaterally stimulating) interactions occur. These observations indicate that very light cutaneous stimulation, possibly limited to movement of relatively few hairs on just one flank, is sufficient to trigger the lordosis reflex of a receptive female hamster, when such stimulation is supplied by a male hamster. Furthermore, the lordosis reflex elicited may not significantly intensify with more vigorous stimulation occurring later in the mating encounter.

Stimulation applied by the experimenter to the back, flanks, rump, or perineum of female hamsters also triggers lordosis Pfaff, Lewis, Diakow and Keiner, 1973; Kow, Malsbury, and Pfaff, 1976). In rats, manual stimulation covering virtually all of the flank, back, tailbase and perineum are required to reliably elicit lordosis (Pfaff, et al., 1973). Stimulation applied to only small portions of this wide area trigger lordosis in hamsters, and hamsters lordose in response to unilaterally applied stimulation

(Pfaff, et al., 1973). Pressure at a "point" with the fingertip on the back, flank or perineum reliably triggers lordosis in estrus female hamsters (Pfaff, et al., 1973). Brushing the hairs of small patches of fur unilaterally within the same area, without exerting pressure, can elicit lordosis from estrogen-progesterone treated ovariectomized females as reliably (100%) as a mating male hamster can (Kow, et al., 1976). Both of these studies indicate that 100% of the females tested showed lordosis in response to unilaterally applied stimulation of the flank alone - no perineal stimulation was required to trigger lordosis. The "threshold" for triggering lordosis in hamsters, then, cannot be gauged if pressure is applied at a "point" or if larger patches of fur are brushed without pressure. Thus, in marked contrast to the rat, unilateral stimulation involving only hair deflection over a very small area of skin not including the perineum, may trigger lordosis in receptive female hamsters.

Noble (1973) has established that prior exposure to a male hamster potentiates the duration of the female's lordosis triggered by manual stimulation applied just after such exposure. This exposure need not include mounting, direct perineal stimulation, or even contact by male in order to potentiate the duration of lordosis (Noble, 1973a; 1973b). The manual stimulation applied by Noble, however, included perineal stimulation, which can elicit additional lordosis reflex components not required to trigger the reflex (see Chapter 1). Although Noble (1973b) reports a significant potentiation of lordosis duration in responders, he does not specifically

comment on the influence male exposure has on the number of females which will respond to manual stimulation. Noble (1973b) does report, though, that manual stimulation elicits lordosis in 73 to 100% of females previously exposed to males, whereas only 53% respond without such exposure. The goal of this study is to establish the effectiveness of some simple forms of light cutaneous stimulation in triggering the lordosis reflex. By judging the intensity of the lordoses elicited it will also be possible to determine whether the lordosis reflex is elicited as a relatively all-or-none motor complex, or as a graded response related to the intensity or total area of applied stimulation.

By applying stimuli before and just after exposure of the test female to a sexually active male hamster, it may also be possible to determine whether the multi-modal stimulus complex supplied by the male facilitates triggering of the lordosis reflex by light cutaneous stimuli.

METHODS

Animals

Adult female golden hamsters (Mesocricetus auratus, outbred LVG strain) were obtained from the Lakeview Hamster Colony (Charles River, Newfield, New Jersey). Hamsters were maintained singly in solid-bottomed rack cages containing sawdust and Kleenex tissue suitable as bedding and nest material. The daily light/dark cycle of the animal room was reversed, 14 hours on/10 hours off; air

temperature was maintained at 19±2°C. Hamsters were allowed ad libitum access to sunflower seeds, guinea pig chow, and rat chow within their home cages. This dry diet was supplemented with fresh cabbage and/or carrots daily.

General experimental history.

Fourteen female hamsters were bilaterally ovariectomized under halothane anesthesia three weeks prior to pretesting. Surgical access to the ovaries was accomplished through a single small skin incision and small bilateral muscle incisions to minimize cutaneous denervation. Three pretests were scheduled to determine that the animals had been sensitized to hormone replacement. All females showed strong lordotic responses to some form of cutaneous stimulation on at least one pretest. Five experimental testing sessions were then scheduled.

On day 1, one hour before lights ON, 10μg estradiol benzoate (EB) in 0.1 cc sesame oil was administered to each animal, subcutaneously. On day 3, (test day), one hour before lights OFF, (41 hours post-EB) 0.5 mg progesterone (P) in 0.1 cc sesame oil was administered subcutaneously. Application of test stimuli commenced 5 hours post-P and all animals were tested prior to 9 hours post-P (two hours before lights ON). This injection and testing regimen was repeated at weekly intervals for the three pretests and five experimental testing sessions. All tests were carried out under normal room illumination.

Testing protocol.

A specific sequence of stimulus sets was begun after placing a female in the center of a 30 cm. circular wooden testing platform and allowing a 30 second habituation period. In general, a set of test stimuli, comprising a series of discrete uniformly applied stimuli presented at a rate of about two per second was administered until the initiation of a lordosis response by the test female. The number of stimuli needed to elicit the lordosis response was recorded and ten further stimuli were applied in the same rhythm. The maximal lordosis intensity achieved during this final stimulation period was qualitatively scored. The female was then bodily picked up, set down firmly on her dorsal rump surface and allowed to regain a normal standing posture. This procedure is necessary to interrupt the typically sustained lordosis response of the female hamster and allows the assumption of a more behaviorally "neutral" posture prior to the application of the next set of test stimuli. If no lordosis occurred during the administration of some set of 40 test stimuli, the female was picked up and set down as before and the next set of stimuli was applied. During the two-minute exposure to a sexually active male, the latency to initiation of the first lordosis response was recorded and the maximal intensity of lordosis achieved during the two minute period was qualitatively scored.

Qualitative assessment of lordosis intensity.

The intensity of lordosis is displayed by a female in response

to any method of stimulation was qualitatively scored by comparison with a complex of motor characteristics observed in the lordosis response to the female hamster.

MINIMAL LORDOSIS female immobile, slight elevation of the rump and tail, back convex, head and facial movement may still be present.

MODERATE LORDOSIS rump elevated so that back is near horizontal and flat, tail held at or near horizontal, head still, eyes fixed, vibrissae slowly waving or stopped, underlying musculature of flank and back firm.

STRONG LORDOSIS rump elevated so that back appears convex, tail near vertical, underlying musculature of sides and back appears taut,stance of rear legs held on toes of feet.

EXTREME LORDOSIS back is deeply convex, tail pointing past vertical, skin along flanks,sides and back is taut over rigid underlying musculature, female is rocked forward in stance so that she almost appears overbalanced over front legs, rear legs rigidly extended with weight on toes.

Thus for each female, a measure of latency to respond, and a measure of response intensity was recorded for each set of test stimuli on each test day.

Characterization of specific test stimuli.

Air puffs. Puffs or jets of air were produced by the rapid manual compression of a large (100cc) rubber pipette bulb. The puffs were directed at the lateral flank surfaces of a test female through the constricted orifice of a plastic eyedropper barrel, from a distance of about 10 cm. The puffs were directed at the hamster from a direction normal to the vertical flank surface of the rear leg. The force of the airstream was sufficient to part both guard and down hairs in a small cone down to the surface of the skin, and the ruffled hairs fell back into place between puffs.

Light brushing. Light brushing of the flank hairs was effected with small, soft bristled camel's hair paint brushes, round in cross section. The brushes were held normal to the surface of the lateral flank and stroked briskly in a caudo-rostrad direction. Brushing was accomplished solely with the soft bristles, and the stroke length was about 5 cm. At each stimulus application the intention was to provide light tactile stimulation and every effort was made to avoid skin indentation and application of pressure to deep structures.

Bilateral brushing. Bilateral brushing was more vigorously applied. The strokes were of the same length, direction, and topography as previous unilateral brushing. Skin indentation and some degree of deep-structure pressure was undoubtably achieved.

Manual stimulation. Manual stimulation was accomplished by the vigorous rubbing and squeezing of the lateral flanks of the hamster between the experimenter's thumb on one flank and fore- and middle-fingers on the other. Considerable deep pressure was applied, and there was also significant contact and pressure on the dorsal rump surface by the ball of the index finger. This was due to the ventro-dorsad sweep of this method of stimulation as opposed to the predominantly caudo-rostrad directed brushing strokes. In general, with all methods of stimulation, the perineal area was scrupulously avoided and stimulation did not extend more rostral than the last few ribs of the thoracic cage.

Exposure to the male hamster. One of the stimulus sets involved the introduction of a sexually active male hamster onto the testing platform. The male was permitted to sniff, lick, bite, manipulate, mount and thrust against the female, but was not permitted to intromit. Thrusting against the perineal surface was frequent, but vaginal insertion was denied. If the male did not initiate active investigation and manipulation of the female within one minute, he was held draped across the back of the female for the rest of the exposure period.

The stimulus sets were presented in the following order on the five test days:

	TEST 1	TEST 2	TEST 3, 4, 5.
1)	2-min. with male	air puffs, one flank	air puffs, one flank
2)	bilateral brushing	air puffs, other flank	air puffs, other flank
3)	brushing, one flank	2-min. with male	2-min. with male
4)	brushing,other flank	bilateral brushing	air puffs, one flank
5)	air puffs, one flank	brushing, one flank	air puffs, other flank
6)		brushing, other flank	brushing, one flank
7)		air puffs, one flank	brushing, other flank
8)		air puffs, other flank	bilateral brushing
9)		manual stimulation	manual stimulation

RESULTS

Two measures of lordotic responsiveness were recorded -- a measure of response latency, and a measure of response intensity. In less than 5% of the trials with discretely applied stimuli (air puffs, unilateral and bilateral brushing) did a test female respond to the stimulus for the first time after ten stimuli had been applied without response. The first stimulus applied triggered lordosis in 38% (169/440) of these trials. With these constraints on the distribution of response latencies, formal analysis was carried no further.

The distribution of response intensities across stimulus types was further characterized. As noted previously, the various stimulus sets were administered in slightly different order on different test days. Application of a particular type of stimulus (e.g. unilateral brushing) in different sequential relation to other post-male stimulus sets did not affect the fraction of females responding on different test days. Therefore all results were pooled across test days, within type of stimulation. The exception to this equivalence with regard to order was air puff stimulation delivered before, as opposed to after the period of exposure to a sexually active male. These two distributions are examined separately.

Figure 2-1 summarizes the distributions of response intensity to type of stimulation pooled across test days, females, and flank laterality. Characteristics of each of these distributions are

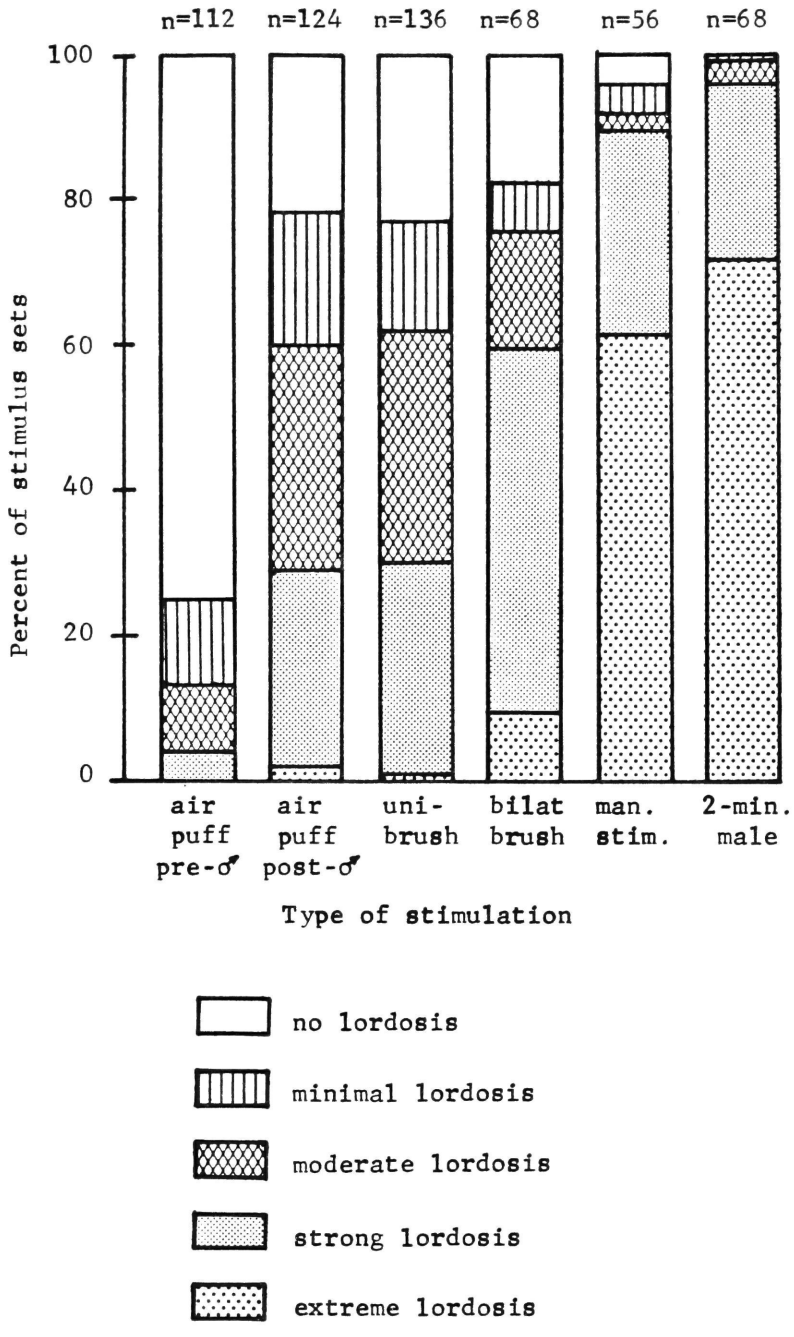

Figure 2-1. Distributions of lordosis response intensities following various types of stimulation. Within each distribution the responses have been summed across test days (n = 5), females (n = 14) and flank laterality (2 for unilateral stimuli).

considered individually below.

Air puff stimulation preceding male exposure. The fraction of females responding to a series of unilaterally applied air puffs delivered prior to exposure of the female to a sexually active male was small on each test day (46%, 18%, 4%, 36%). This result is reflected in Figure 2-1, which indicates the fraction of air puff stimulus sets (summed across flank laterality, females, and test days) effective in eliciting lordosis from the female hamsters. Half of the females (7/14) never showed lordosis on any of the five test days, and half of all lordoses observed were of minimal intensity.

Air puff stimulation after male exposure. When air puffs were applied following the ministrations of a sexually active male hamster, lordosis responses were apparent in 78% of the tests (see Figure 2-1). In fact, every female showed lordosis in response to air puffs on at least one test day. Eight of fourteen females showed lordosis on every test day, and 76% (74/97) of all lordoses elicited were moderately or more intense. The number of females responding to unilateral air puffs after male exposure is significantly increased over the number responding before male exposure, at the $p < .001$ level (McNemar test, $x^2 = 56.0$, df = 1).

Brushing. There is no apparent difference in the efficacy of unilateral as opposed to bilateral brushing stimuli in eliciting lordosis(both follow male exposure). Indeed, the percentage of tests in which lordosis was elicited was very similar for post-male air puffs (78%), unilateral brushing (77%), and bilateral brushing (82%).

Bilateral brushing did elicit more intense responses than either unilateral stimulus (see below). Of lordoses observed in response to unilateral stimulation, 36% and 39% (post-male air puffs 35/97, unilateral brushing 41/105) were scored as strong or extreme. With bilateral brushing 71% (40/56) of all responses observed were scored strong or extreme.

Manual stimulation. Vigorous manual palpation of the posterior flank and rump areas was very effective in eliciting lordosis responses (see Figure 2-1). On only three of 56 applications of manual stimulation did the test female fail to respond by assuming a lordotic posture, two of these exceptions originating with the same female. Of the lordoses seen to accompany manual stimulation 94% (40/53) were scored strong or extreme.

Exposure to a male hamster. Interaction with a sexually active male hamster uniformly elicited lordosis responses from the test females. No female failed to show lordosis on any test day, though the male was held draped over the females' rump in six tests when no lordosis had occurred after 1 minute of exposure. Ninety-six percent (65/68) of the lordoses observed were scored strong or extreme.

Comparisons of lordosis response intensity to artificially applied stimulation.

The lordosis intensity data in response to different types of stimulation were compared using the McNemar test for the significance of changes, corrected for continuity (Siegel (1956) Nonparametric Statistics p. 64). Lordosis responses to post-male unilateral air

puffs were significantly more intense than the lordosis responses to pre-male unilateral air puffs (chi square = 8.5, df = 1, $p < .01$). This comparison of samples matched by female and flank did not include those tests in which pre-male air puffs did not elicit lordosis.

The intensity of lordosis responses to unilateral brushing and bilateral brushing were also compared. Again, the statistic was used conservatively - the more intense response to unilateral stimulation (right flank or left flank) was compared to the response to bilateral brushing, and tests on which no response occurred to unilateral brushing were excluded. Bilateral brushing triggered significantly more intense lordosis responses than unilateral brushing (chi square = 18.4, df = 1, $p < .001$).

The intensities of lordosis responses triggered by manual stimulation were compared to the lordosis responses triggered by bilateral brushing. Again, application of the McNemar test was conservative. The direction of change in response intensity was included only if bilateral brushing triggered a response. Manual stimulation elicited significantly more intense lordosis responses than bilateral brushing (chi square = 38.0, df = 1, $p < .001$).

DISCUSSION

In considering the results of this study it is important to distinguish between the elicitation of a lordotic response of any intensity, and the final intensity of a response triggered by some set of cutaneous stimuli. As the description of MINIMAL lordosis indicates, not only is the elevation of the rump and head less pronounced, but other components of the complete motor complex are not displayed at all. Once a response attains the criteria for MODERATE lordosis, all components are present, though some may be weakly expressed. STRONG lordoses are typical response intensities observed in response to sexual stimulation by the male, and most receptive females attain EXTREME response levels at some point during a mating sequence. Thus, the fraction of females responding with lordosis of at least MINIMAL intensity provides an index of the probability that similar stimuli will trigger lordosis responses in receptive females. Cutaneous stimuli eliciting MINIMAL lordotic responses, however, do not appear to activate neural circuitry controlling some facets of the motor complex sufficiently to observe behavioral changes. Once MODERATE response levels have been achieved, all relevant motor control circuits appear at least minimally active. Responses at STRONG or EXTREME levels indicate not only a fully active lordosis motor integration, but also intense activity throughout the efferent system expressing the reflex.

Bearing these points in mind, consider the elicitation of lordotic responses by gentle puffs of air, delivered unilaterally,

either before or after exposure of the test female to the ministrations of a sexually active male. Before such exposure, air puffs elicited lordotic responses in about one quarter of the trials, and half of the lordoses observed were minimally intense. After male exposure, air puffs elicited lordosis in three-quarters of the trials and three-quarters of these responses were at least moderately intense. The period of sexual interplay has not only significantly facilitated the subsequent triggering of the lordosis reflex, but also potentiated the intensity and completeness of the motor response. There is no indication that this facilitation and potentiation is especially rapidly decaying. The response distributions of the first two test days when post-male air puffs were delivered after several intervening stimulus sets were not different from the distributions of responses on the last three test days when post-male air puffs were delivered immediately following the male exposure period.

These results show that light cutaneous stimulation, exerting very little pressure at a relatively small "point" on the flank, can trigger lordosis in female hamsters. However, this lightly applied stimulation (air puffs) is not usually effective, and generally elicits low intensity lordosis responses. As reported by Noble (1973b) prior exposure to a male hamster potentiates lordosis responding to artificially applied stimulation. Furthermore,lordosis was facilitated by this exposure -- significantly more air-puff tests

triggered lordosis following male-exposure. This effect did not require the continued presence of the male during the air-puff tests. The facilitation and potentiation by prior exposure to the male are seen without application of perineal stimulation in the post-male test, so these effects do not depend on the triggering of rump displacement reflexes by perineal stimulation (see Chapter 1).

Both unilateral air puffs and unilateral brushing were administered after the male exposure period, and the response distributions to these two forms of stimulation are quite identical. The area stimulated by post-male bilateral brushing was at least twice as large as the area stimulated by either unilateral method of stimulation. Also the strokes were more vigorously applied. Yet, bilateral brushing was no more effective in triggering lordosis than the application of unilateral stimuli. The responses to bilateral brushing were significantly more intense than those to unilateral stimuli, and the response to manual stimulation was significantly more intense still. Thus, light stimulation can trigger the complete lordosis reflex as reliably as stronger stimulation, but stimuli covering more area, or more vigorously applied, trigger more intense responses. As "artificial" stimulation mimicing the topography of natural stimulation becomes even more intense and widely applied, response intensity eventually equals that elicited by the male hamster. The response distribution resulting from vigorous manual palpation of the flanks and dorsal rump differs very little from that resulting from the male exposure period. The force of applied manual stimulation certainly

exceeds that supplied by the male, but it is important to note that manual stimulation can, if only at intense levels, fully mimic the male hamster in triggering intensely displayed lordotic responses. Furthermore, manual stimulation need not include a perineal component in order to equal the potency of mating stimulation.

CHAPTER 3

SELECTIVE BRAINSTEM TRANSECTIONS AFFECTING REPRODUCTIVE BEHAVIOR OF FEMALE RATS: THE ROLE OF HYPOTHALAMIC OUTPUT TO THE MIDBRAIN.

INTRODUCTION

In rodents, reproduction depends on the fertilization of ova within the reproductive tract of the female. Consequently, the mating behavior of the female must, at some point, permit the male to achieve the required intromission. During copulation, female rodents allow this access by adopting a stereotyped posture which presents the vaginal opening in a favorable position for intromission to occur. This stereotyped posture is called lordosis, and the female adopts this stance as a reflexive response to the mount of the male. Lordosis can also be triggered by manual stimulation, supplied by an experimenter as an imitation of the copulatory mount of the male.

The lordosis reflex of female rats has been studied by film (Pfaff and Lewis, 1974) and X-ray (Pfaff, Diakow, Montgomery & Jenkins, 1978) cinematography. These studies have shown that lordosis is a stationary, standing posture involving a deep vertebral column dorsiflexion, with marked elevation of the rump and tailbase, depression of the thorax, elevation of the head, and extension of the legs.

This behavior is shown by females only during that period of the estrus cycle which coincides with the maturation of ovarian follicles and ovulation. At other times, females reject mounting attempts by a male. This synchrony of receptive behavior with the estrous cycle insures that mating will only take place while the female is fertile. At this point in the estrous cycle, circulating estrogen levels have been high for some time, and progesterone levels are rising (Yoshinaga, et al., 1969; Hasimoto, et al., 1968). If the primary source of these steroids is removed by ovariectomy, lordosis does not occur. Replacement of

estrogen alone in ovariectomized females fully restores lordosis behavior (Davidson, et al., 1968; Pfaff, 1970). Estrogen-dependent lordosis behavior is facilitated by progesterone (Beach, 1942).

Although many reflexes are fully integrated by the spinal cord and lower brainstem, this is not the case with the estrogen-dependent lordosis reflex. Kow, Grill and Pfaff (1978) have shown that some part of the forebrain is required for lordosis behavior. Following separation of the forebrain and lower brainstem by complete transections just rostral to the superior colliculus, estrogen primed, ovariectomized female rats did not show lordosis. Lordosis was eliminated in both mating tests with male rats, and in manual stimulation tests when perineal stimulation was supplied. These decerebrations were completed in two steps. One vertical hemisection was made, and two weeks later a second vertical hemisection completed the decerebration. No deficit in lordosis resulted from the initial hemisections. Thus Kow, et al (1978) were not only able to show that complete transections at this level eliminate lordosis, but also that the critical fiber systems can fully integrate and sustain lordosis behavior after a vertical hemisection. Control transections were made in the same coronal plane, but penetrating only the dorsal pre-tectum. These control transections left most of the brainstem intact, and no deficits in lordosis were seen after transection.

Complete transection studies prove that some pathway between the forebrain and lower brainstem is required for lordosis to occur, but do not indicate which of the interrupted fibers participate in the control of lordosis. In fact, from this transection strategy the effect cannot be attributed to transection of an ascending as opposed to a descending system, nor can the requirement for intact two-way communication

between the forebrain and lower brainstem be ruled out. In the DISCUSSION section, evidence is reviewed which argues against the transection of fibers ascending through this level accounting for this effect, or playing a crucial role in the control of lordosis. However, a specific forebrain cell-group, which contributes fibers descending through this level has been implicated in the control of estrogen-dependent lordosis behavior. This cell-group is the ventromedial nucleus of the hypothalamus.

Participation by VMN of the hypothalamus in control of lordosis.

Several laboratories have reported that bilateral lesions involving the ventromedial nucleus (VMN) of the hypothalamus severely reduce the number of successful copulations between male and female rats (e.g.Averill and Purves,1963;Kennedy,1964;Kennedy and Mitra,1963;Carrer,Asch,and Aron, 1973). Furthermore, Kennedy (1964) documented this effect in ovariectomized, estrogen-progesterone replaced females, indicating that the lesion effect in otherwise intact females could not be solely attributed to disruption of pituitary-gonadal function. These studies relied on indirect (presence of sperm in vaginal smears or vaginal plugs) evidence of mating behavior. Mathews and Edwards (1977) and Pfaff and Sakuma (1978b) have recently extended the behavioral analysis of this VMN lesion effect by reporting on direct observations of lordosis behavior by ovariectomized, hormone-replaced rats. Mathews and Edwards (1977) showed that bilateral lesions involving at least the medial VMN always abolished lordosis in mating tests following replacement of estrogen alone. This effect disappeared when progesterone was supplied in addition to estrogen.

Pfaff and Sakuma (1978b) confirmed this VMN-lesion effect in estrogen-replaced ovariectomized females. They showed that the decline in lordosis behavior is gradual, typically requiring 36 - 60 hours to reach its maximum. This minimal level of response recovers, but not to pre-operative levels, two weeks after lesioning. Pfaff and Sakuma (1978b) also demonstrated this deficit when lordosis was tested by manual palpation - indicating that a failure on the part of male rats to deliver perineal stimulation cannot account for the deficits in the mating tests.

Pfaff and Sakuma (1978a) have provided further evidence that the VMN plays a tonic, facilitatory role in the control of lordosis by testing for the effects of electrical stimulation at the VMN sites which, when lesioned, lead to lordosis deficits. Low intensity, low frequency electrical stimulation in or very near the VMN facilitates lordosis in ovariectomized, estrogen-primed female rats (Pfaff and Sakuma, 1978). This facilitation is seen after a period of 15 - 60 minutes of stimulation and can last 5 - 8 hours after the termination of stimulation. The effect requires pre-treatment with estrogen, but not progesterone. Furthermore, unilateral VMN stimulation is sufficient to facilitate lordosis.

During the estrus cycle, the circulating concentration of estrogen varies. Ventromedial nucleus cells concentrate estradiol from the blood (Pfaff and Keiner, 1973) and more VMN neurons are electrically recordable following systemic estrogen treatment (Bueno and Pfaff, 1976). The functional significance of this estrogen uptake for lordosis has been investigated by implanting estradiol in the ventromedial nucleus. Such

implants can allow ovariectomized females to show lordosis, without other hormone replacement (Barfield and Chen, 1977; Dörner, Docke, and Moustafa, 1968). When ovariectomized females are tested in conjunction with progesterone replacement, smaller estrogen implants near the VMN are sufficient to "prime" the lordosis control mechanisms. Progesterone-treated, cholesterol-implanted control rats do not show lordosis. The amount of VMN-implanted estrogen required for this "priming" effect is so small that diffusion to other forebrain structures may be ruled out, and the performance of lordosis can be attributed to the local influence of estradiol on VMN cells (Davis, et al., 1979).

VMN efferents to the lower brainstem.

These functional studies allow the hypothesis that axons descending from the ventromedial nucleus to the lower brainstem are critical to the estrogen-dependent control of lordosis. Recent anatomical work (Krieger, et al, 1979) has defined the efferent projections of the ventromedial nucleus, and shown that the VMN contributes long, descending projections which reach the midbrain, pons and medulla.

Fibers exit the VMN and descend along two distinct trajectories (Krieger et al, 1979; shown in Fig. 3-1 and 3-2). Some VMN fibers fan laterally as they leave the nucleus from the side (Fig. 3-1, sec. E), and after reaching a lateral position in the brainstem (Fig. 3-1, sec. F) turn caudally and descend along a lateral pathway (Fig. 3-1 and 3-2, secs. F through J). This lateral projection rises dorsally as it descends through mammillary levels, some fibers turning medially to distribute within and lateral to the central grey (Figs. 3-1 and 3-2,

sec. G through J). At the level of the red nucleus, nearly all of these fibers are contained in the dorsal half of the brainstem (Fig. 3-2, sec. I) and by anterior pontine levels (Fig. 3-2, sec. J), the fibers continuing to descend are located within and lateral to the central grey, in a more medial trajectory.

Another set of fibers descends from the ventromedial nucleus along a medial, or periventricular trajectory. These fibers exit the VMN medially, and begin to rise dorsally as a diffuse sheet, rising to the central grey and continuing to descend by mammillary levels (Fig. 3-1, secs. F,G). This medial pathway continues to descend in the central grey (Fig. 3-2, sec. H) and by red nucleus levels is contained in the dorsal half of the brainstem (Fig. 3-2, sec. I). At pontine levels, fibers continue to descend in and lateral to the central grey and are not separable from the fibers reaching this level by the lateral pathway through the midbrain (Fig. 3-2, sec. J through L).

The present study is designed to determine which of these fibers descending from the ventromedial nucleus are essential in the control of estrogen-dependent lordosis. Both the medial and lateral projections descend as sheets of fibers. Therefore, a strategy of selective transections was chosen to interrupt the medial and lateral pathways, separately and together, as they descend to reach the lower brainstem.

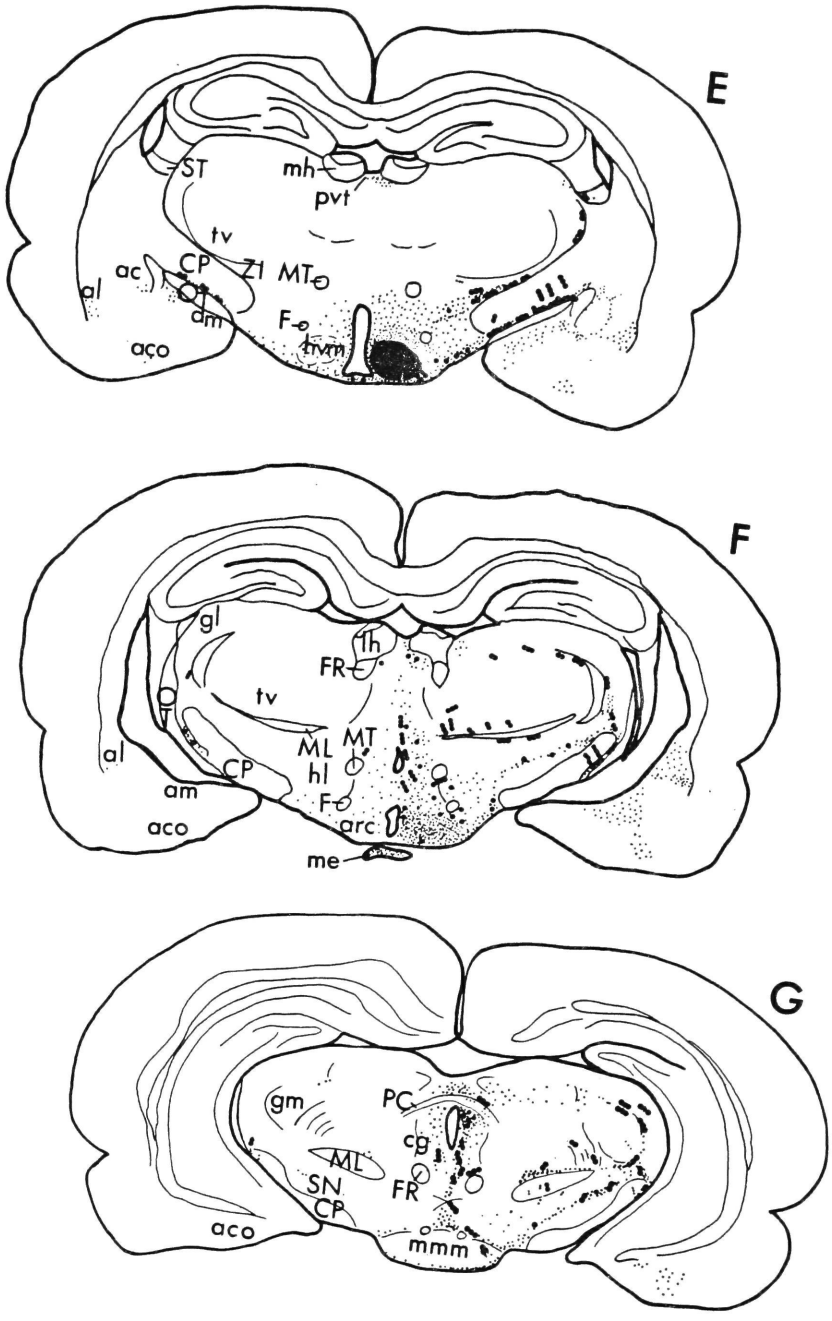

Figure 3-1. Distribution of reduced silver grains following autoradiographic exposure. Injection site (solid black,E) coextensive with ventromedial nucleus of hypothalamus (hvm). Large dots represent labeled axons; small dots, fields of terminals or small unmyelinated axons.
From: Krieger, Conrad and Pfaff, 1979.

Figure 3-2. More caudal sections, same brain as Figure 3-1.

METHODS

Adult Sprague-Dawley female rats (Hormone Assay Lab), ovariectomized by the supplier were used. All rats were housed singly, with commercial chow and water available ad. lib. The light-dark cycle of the colony room was reversed (12 light: 12 dark) with lights off at 9:00 AM. After arrival, rats were given at least two weeks to adapt to the controlled colony environment and attain body weights between 250 and 350 grams. During this period, manual stimulation tests for lordosis were administered and vaginal smears taken. These tests established that ovariectomy had eliminated periodic receptivity and estrus cycling.

Each rat was then given an estrogen-containing implant subcutaneously at the nape of the neck. Implants consisted of a short length of silastic tubing (0.58 in I.D. x 0.77 in O.D.). Crystalline estradiol 17-B was packed into this tubing to a length of 10 mm, and the ends were sealed with silastic adhesive.

Beginning two weeks after estrogen replacement each female was tested for lordosis behavior on three or more pre-operative days, at least 5 days apart. On each test day each female was given a manual stimulation test just before, and just after a mating test with a vigorous stud male rat (see test descriptions below). Only those rats showing high, consistent lordosis test results pre-operatively were used for further experimentation. Rats were used in 8 squads over a two year period. Within each group of animals qualifying for surgery, rats were randomly assigned to the various categories of selective transections.

Manual stimulation tests; the lordosis reflex score.

In manual stimulation tests, the experimenter's hand mimics the stimulation applied to the female by the male's mount. Several brushing strokes are applied to the female's flanks, then the female is grasped with two fingers forked around the tailbase to exert pressure on the perineum. The rest of the hand grips across the female's rump allowing the thumb on one side, and the ring and little fingers on the other side, to contact the female's flanks. When squeezed, this "fork" grip exerts pressure on the rump, flanks, and perineum closely resembling the pattern of stimulation applied by the male during copulation (Kow, et al., 1979). In addition, manual stimulation insures that the female receives perineal stimulation, which cannot be applied by the male unless the female lordoses.

The response of the female to manual stimulation was recorded as the "lordosis reflex score" very similar to that employed by Kow, et al. (1977). The lordosis reflex score is a sum of four measures:

(A) Intensity of manual stimulation needed to trigger the lordosis reflex (light touch scores 3; moderate pressure, 2; substantial pressure, 1; hard squeeze, 0).

(B) Maximal intensity of rump elevation felt by experimenter during manual stimulation (strong, 3; moderate, 2; weak, 1; none, 0).

(C) Maximal elevation of head during manual stimulation (head tipped up and back more than 45° above horizontal, 3; head between horizontal and 45°, 2; head lifted but not above horizontal, 1; head not lifted, 0).

(D) Tailbase elevation (raised, 1; not raised, 0).

Thus lordosis reflex scores range from 0 to 10 (no lordosis to strong squeeze = 0; extreme lordosis to light contact = 10). Thus the lordosis reflex score is a compound score indicating: (1) the approximate pressure threshold at which the response is triggered, as gradually increasing manual stimulation is applied with the "fork grip"; and (2) the maximal intensity of any lordosis reflex triggered during the manual stimulation test.

Mating tests; lordosis quotients.

Mating tests were conducted in lens-shaped testing arenas with vertical plexiglass walls (maximally 75 cm x 45 cm x 20 cm deep). The floor was textured, and covered with sawdust. One male rat was placed in each of several arenas, and test females were shuttled from one male to the next as necessary to expose the female to 15 vigorous mounts.

The responses of the female were recorded on a seven-point scale to each mount or mount attempt (both paws clasp female) by the male:

(-3) = female behavior prevents male mount (fighting, rolling on back).

(-2) = female behavior dislodges male from mount (running, kicking).

(-1) = female continues ongoing behavior but does not prevent male from completing copulatory mount.

(0) = female assumes immobile posture, but no lordosis.

(1) = poor or fractionated lordosis, maximal dorsiflexion reaches only "flat back".

(2) = normal lordosis, dorsiflexion deeper than flat, head above horizontal.

(3) = extreme lordosis, extreme dorsiflexion, head elevated above 45° from horizontal.

These female responses were recorded for a minimum of 15 vigorous thrusting mounts (including intromissions and ejaculations) in each mating test. These scores were used to calculate the lordosis quotient, (number of lordoses shown)÷ (number of thrusting mounts by male) x 100 = LQ. Thus the lordosis quotient is an index of the probability that a vigorous mount by the male will trigger a lordosis response (of any intensity 1, 2 or 3) by the female.

Surgery.

Transections were performed on animals anesthetized with halothane. The rat's head was held in a standard stereotaxic device. The skull was exposed and appropriately located slots were drilled through the skull. The exposed dura was cauterized and cut. The transection knife was then lowered into the brain and moved with a micromanipulator to effect the intended transection. The knife was removed and the bilaterally complimentary transection effected,as desired. The surface of the cortex was monitored until bleeding had apparently stopped, and the scalp incision was closed with sutures. Transection knives were ground from stainless steel scalpel blades to a thickness of 0.15 mm and shaped suitably for the intended transection. L-shaped transection knives were used to reach the midline without rupturing the blood-filled mid-sagittal sinus lying

on the top of the brain. Other transections (bilateral lateral, parasagittal) were made with straight transection knives.

The transections were placed with reference to the sutures between the bones of the dorsal surface of the skull. The rat's head was fixed between the earbars of the stereotaxic device, defining the interaural line. The dorsal surface of the skull was then brought into "level" by adjusting the elevation of the bite bar relative to the earbars. The "head level" position was determined by bringing the intersection points of the transverse sutures (lambda and Bregma) with the mid-sagittal suture to the same elevation above the interaural line. All transections were then made perpendicular to this "head level" plane. The depth of every transection was measured down from the surface of the dura overlying the cortex, where it was exposed nearest the midline. The rostrocaudal placement, and extent, of each transection was determined in coordinates relative to the Bregmoid and mid-sagittal sutures, as follows:

1) Dorsal hemisections were 10 mm wide, centered on the mid-sagittal sinus; extending 6 mm. deep to the dura. Thalamic hemisections were located in a coronal plane 3 to 4 mm. posterior to Bregma. Dorsal hemisections at the level of the red nucleus were located in a coronal plane. 5.7 to 6 mm. posterior to Bregma.
2) Cortical transections were 10 mm wide, centered on the mid-sagittal sinus; extending 3.5 mm. deep to the dura. They were located in a coronal plane 4 mm. posterior to Bregma.

3) Medial transections were 4 mm. wide, centered on the mid-sagittal sinus. Shallow medial transections extended 6 mm. deep to the dura; deep medial transections extended 6.5 or 8 mm deep to the dura. Medial transections were located in a coronal plane 5.7 (shallow), 6.0 (deep, red nucleus) or 6.5 (deep, posterior to red nucleus) mm. posterior to Bregma.

4) Parasagittal transections were located in vertical planes parallel to, and 1.2 or 3.0 mm. lateral to, the mid-sagittal sinus. The transections extended 7.5 or 8.0 mm. deep to the dura. The rostral end of the transection was located 1.5 mm. posterior to Bregma, extending caudally 3.0 mm. to a caudal termination 4.5 mm. posterior to Bregma.

5) Lateral transections were 2.0 mm. wide, and located in a coronal plane. The medial edge of each transection was located 2.0 mm. lateral to the mid-sagittal sinus. The plane of transection was located 5.8 to 6.5 mm. (deep bilateral, shallow bilateral, unilateral), or 7.5 mm. (mesencephalic-pontine junction bilateral lateral) posterior to Bregma. The transections extended 6.0 mm. (shallow), or 7.0 to 8.0 mm. (deep) deep to the dura.

A total of 175 rats were transected, and 65% of these animals died immediately following surgery or shortly thereafter. Post-mortem dissection revealed extensive bleeding within the skulls and brains of many of these animals. This blood loss, or the local effect of bleeding and fluid pressure within the brain may account for the death of many of these animals. Other brains did not show evidence of heavy bleeding

and in these cases, death may have been due to the severing of certain vital neuronal pathways (e.g. hypothalamic fibers important for autonomic function).

Post-operative observations and testing.

On days 1 through 5 post-op, transected females were given manual stimulation tests daily. Thereafter, manual stimulation tests were administered both before and after each mating test. Mating tests were about one week apart throughout the post-operative period. All animals were allowed to survive for at least 3 mating tests (about 3 weeks, minimum). Body weight, body temperature and excretory function were noted daily until the first mating test, and then in conjunction with each mating test. Animals failing to feed on chow and/or cookies in sufficient amounts to maintain body weights were fed 15 mls. of a vitamin supplemented milk diet twice daily by intro-gastric intubation (Kissilieff, 1972).

Three or more mating (and the accompanying manual) tests were administered post-operatively to assay the lordosis performance of transected animals. These tests were distributed over at least three weeks to allow recovery from non-specific surgical trauma. When these tests indicated a stable level of post-operative performance hormonal manipulations were made on some animals.

Some animals received estradiol benzoate, or progesterone, or both, in supplement of ongoing estrogen replacement by implant. Estradiol benzoate (EB) was given by daily injections of 10μg EB/0.1 ml

sesame oil, subcutaneously for at least 3 days prior to a mating test. Progesterone was given at 0.5 mg/0.1 ml sesame oil, also subcutaneously, about 6 hours before a mating test. Such a course of EB or EB&P allows lordosis responses to be reliably elicited from ovariectomized females not receiving ongoing estrogen replacement by implant. Other animals had their estrogen-containing implants removed, and were tested 1 to 2 weeks later.

In conjunction with post-operative lordosis testing, each animal's responses to simple neurological tests were noted. These tests included pinches to the extremities and pinnae, righting from a supine position and careful notation of deficits or changes in resting posture or gross locomotory pattern.

Histological reconstruction of transections.

At the end of the post-operative period, a vaginal smear was taken to confirm high estrogen replacement levels, and the patency of the estrogen-containing silastic was confirmed visually. After post-operative testing was completed, each rat was overdosed with Nembutol, perfused transcardially with 10% formalin and the brain was removed. Brains were stored first in 10% formalin, transferred to 30% sucrose - 10% formalin, then frozen and sectioned at 100μ on a microtome. The plane of histological section was perpendicular to the plane of transection - sagittal for coronal transections; coronal for parasagittal transections. The complete series of sections were stained with Luxol Fast Blue and Cresyl Violet for histological reconstruction of the extent and rostro-caudal placement of each transection.

Histology was prepared in a plane perpendicular to the plane of transection. Therefore, transections appeared as slices through the stained tissue. Figures 3-3 to 3-6 show tracings of microprojections of four sequential sagittal sections through the brain of rat 55. The transection is indicated by a heavy black line, with hatched areas bounded by a heavy black line indicating empty or ablated volumes in the plane of the transection. The consistency of the rostrocaudal placement and depth of the transection is apparent in Figs. 3-3 to 3-6. The transection lies in a coronal plane including the rostral superior colliculus dorsally, the rostral tip of the red nucleus and medial lemniscus medially, and the mammillary peduncles at the level of exit of the third cranial nerve ventrally.

Identification of anatomical landmarks such as these enabled me to chose an appropriate coronal section from a reference atlas (König and Klippel, 1970). I then mapped the extent of the transection from each sagittal section onto the appropriate reference coronal drawing.

For example, the coronal reconstruction of the transection shown in sagittal sections (Figs. 3-3 to 3-6)appears as Fig. 3-7. The four sagittal sections are located just medial to the descent path of the transection knife through the cortex on the left-hand side.

The animals were then grouped according to the similarity in extent and location of their transections, and the lordosis test results for similar animals were compared.

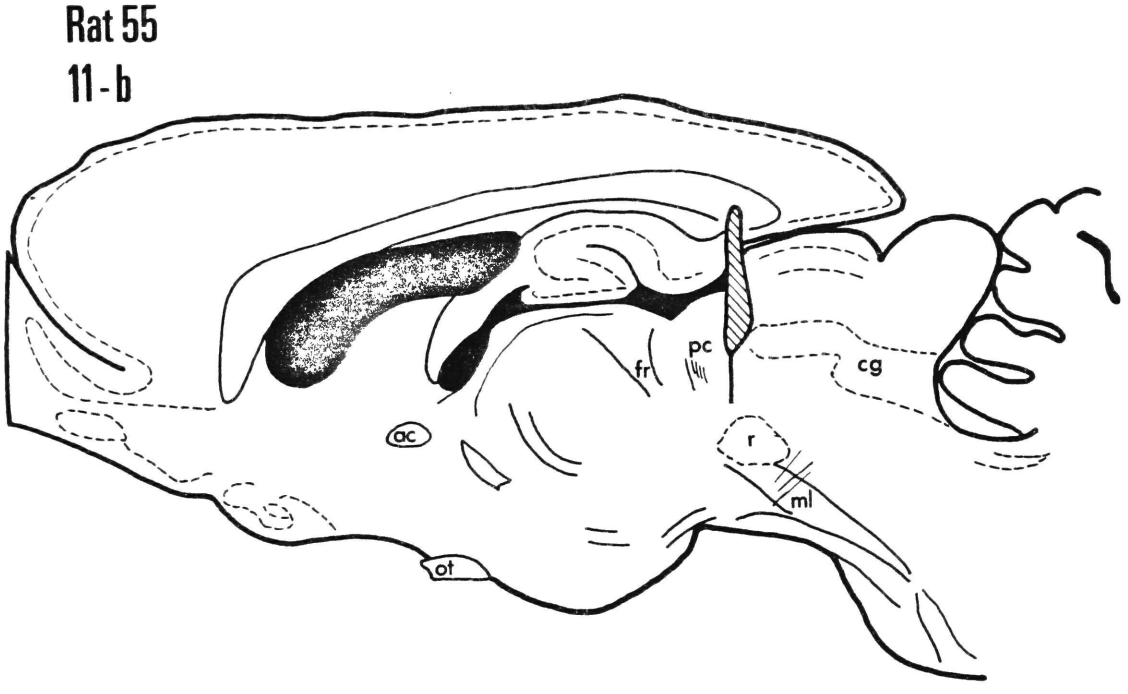

Figure 3-3. Tracing of a microprojection of the first of four sequential 100 μ - thick sagittal sections. Ventricles are colored black. Transection is indicated by a heavy black line. Hatched areas surrounded by a heavy black line indicate empty or ablated volumes as a result of

transection. Unbounded hatched areas indicate degeneration. The following abbreviations have been used in anatomical figures:

ac	anterior commissure
cg	central grey
cp	cerebral peduncle
dm	dorsomedial nucleus
f	fornix
fr	fasciculus retroflexus
h	habenula
ic	internal capsule
ip	interpeduncular nucleus
mb	mammillary body
mg	medial geniculate
ml	medial lemniscus
ot	optic tract
pc	posterior commissure
pv	periventricular nucleus
r	red nucleus
sn	substantia nigra
vm	ventromedial nucleus

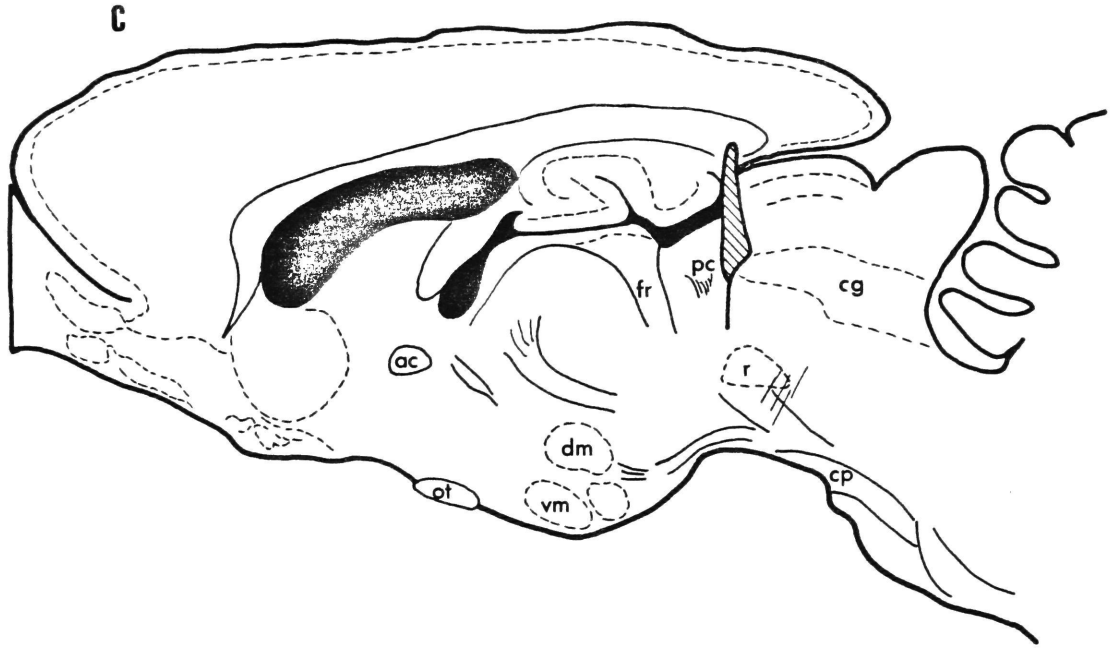

Figure 3-4. Second of four sequential sagittal sections.

Conventions as in Figure 3-3.

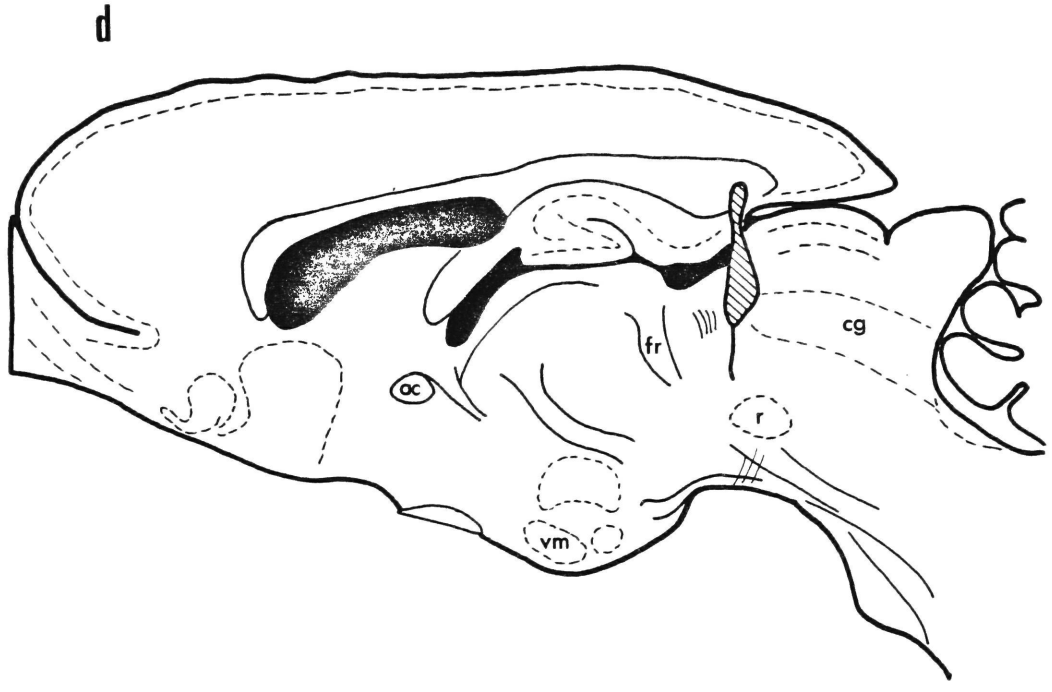

Figure 3-5. Third of four sequential sagittal sections.

Conventions as in Figure 3-3.

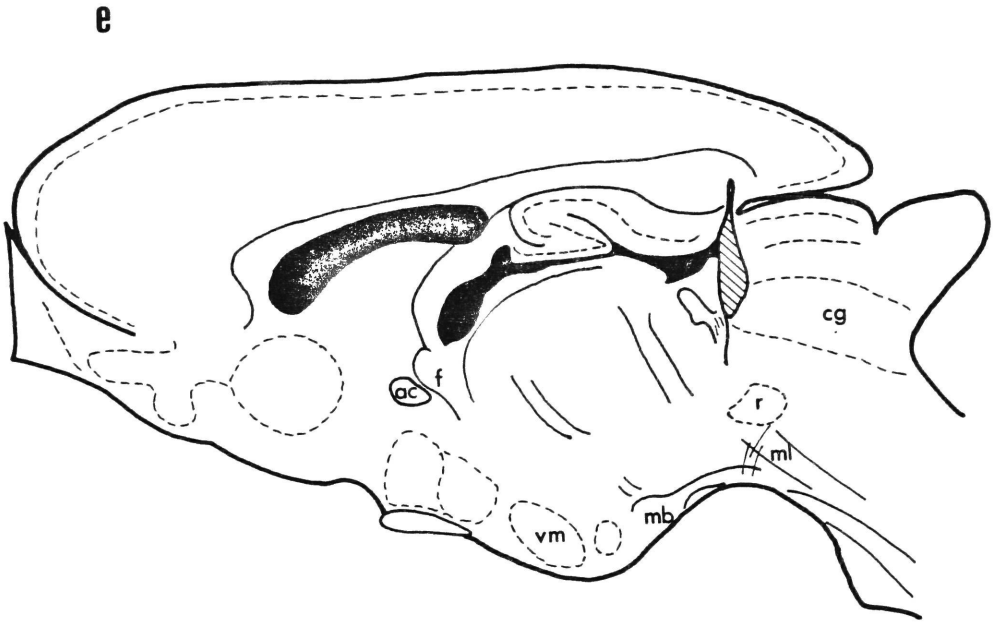

Figure 3-6. Fourth of four sequential sagittal sections. Conventions as in Figure 3-3.

RESULTS

Dorsal hemisections.

Twenty-seven animals received dorsal hemisections of the brainstem at various rostrocaudal levels, and seven of these animals survived chronically. Fibers in both the medial and lateral pathways rise dorsally as they descend from VMN to reach the central grey. Thus rostrally located hemisections will spare most or all of these fibers while dorsal hemisections at the level of the red nucleus will interrupt most or all of each pathway. Therefore, the 7 surviving animals were divided into 2 groups on the basis of the rostro-caudal placement of their hemi-sections. In addition, 2 control animals received cortical transections which did not penetrate to the brainstem.

Dorsal hemisections at the level of the red nucleus: The coronal reconstruction of the transection of rat 55 is shown in Figure 3-7. This transection passes through the rostral superior colliculus, at the level of the anterior red nucleus. It extends fully across the brainstem , to a depth just below the cerebral aquaduct. Rat 55 was strong and healthy following surgery, thermoregulated at a normal rectal temperature, but did not eat voluntarily for the first 2 weeks following surgery. At about 2 weeks post-op, rat 55 resumed eating, and began to show a strong left-beating nystagmus of the eyes and head. At this point the previously normal locomotory pattern became dominated by continuous, tight, counterclockwise circling.

The pre- and post-operative lordosis test results for rat 55 are shown in Figure 3-8. The lordosis reflex score fell to zero the

day following surgery and remained at zero throughout the post-operative period. In post-operative mating tests, male rats could never elicit lordosis responses from this female. This chronic elimination of lordosis was not affected by the administration of EB and P (at dosage levels sufficient to restore lordosis behavior in ovariectomized females) in supplement to ongoing estrogen replacement by the implant. No sign of recovery of lordosis performance was seen 43 days after transection.

Two other animals survived chronically with transections at the level of the posterior red nucleus. The transection in rat 67 extended to the bottom of the cerebral aquaduct on the left, and slightly deeper on the right. The transection in rat 281 nearly reached the dorsal margin of the red nucleus, bilaterally.

Rat 67 maintained a normal body temperature, but did not eat voluntarily following transection. During the last week of the 30 day post-op period this animal showed a strong, right-beating nystagmus, and locomotion was dominated by a pattern of circling in a clockwise direction. Manual stimulation tests were administered daily and rat 67 showed strong lordosis to light contact only on days 2, 3 and 11; otherwise, no lordosis could be elicited during the first two weeks post-op. Less intense lordoses to stronger stimulation were shown reliably during the final 10 days of post-op testing. Rat 67 never showed lordosis in post-operative mating tests without EB and P added to the E replacement by implant. When tested following this additional steroid replacement, rat 67 attained LQ's of only 33 (EB+P) and 27 (P alone) compared to a pre-operative mean of 95.

Rat 281 was active and alert post-operatively, showing no obvious neurological deficits and feeding sufficiently to maintain pre-operative body weight. Pre-male manual stimulation did not elicit lordosis responses until 2 weeks post-op, but post-male lordosis reflex scores were at pre-operative values throughout the 24 day post-op period. Mating tests were administered on days 5, 10, and 15 yielding LQ's of 47, 0, and 0. The LQ following a course of four daily EB injections with P injected 6 hours before the mating test was 20, compared with preoperative LQ's averaging 100.

Summary: Transections of the dorsal brainstem at the level of the red nucleus, can severely interfere with the estrogen-dependent performance of lordosis in response to both mating and manual stimulation. Lordosis in the mating test was nearly eliminated. This deficit could be slightly improved by administration of additional EB and P. Manual stimulation was more effective in triggering the reflex, but some deficit remained throughout the post-operative period.

Dorsal hemisections at thalamic levels: The remaining 4 animals had dorsal hemisections located in the posterior thalamus, just posterior to the habenula, and extending down into the brainstem approximately in the plane of the fasciculus retroflexus. Figure 3-9 shows the coronal reconstruction of the transection in rat 161, representative of this group of 4 animals. These transections also produced considerable degeneration of the dorsal thalamus, rostral to the transection plane itself. All of these animals were healthy and vigorous throughout the post-operative period. They fed and thermoregulated normally. No locomotory or neurological deficits were obvious.

Figure 3-10 shows the history of lordosis test results for animal 161. Recovery of lordosis to manual stimulation was immediate and complete post-operatively. The lordosis quotients in mating tests showed that lordosis was elicited with nearly pre-operative reliability.

The other three animals with similar thalamic hemisections all showed lordosis postoperatively, although the recovery of the response to manual stimulation was not as immediate as with rat 161. One of these rats showed post-operative test results much the same as rat 161 - both measures of lordosis recovered to pre-operative levels. The other two showed mixed results - their lordosis reflex scores were increased relative to pre-op; and their lordosis quotients decreased.

Thus, hemisections at the posterior thalamic level had a mixed effect, or none at all, on lordosis performance.

Cortex only: Two animals served as surgical controls. They received coronal transections limited to the cortex, hippocampus and dentate gyrus overlying the brainstem at the level of the mammillary bodies.

Lordosis performance in male and manual tests was not affected by these control transections, relative to pre-operative performance. (Mean preoperative LQ = 96, post-op = 85; mean pre-operative post-male lordosis reflex score = 9.5, post-op = 9.9). Thus, deficits seen to follow deeper transections penetrating the dorsal brainstem cannot be attributed solely to non-specific surgical trauma.

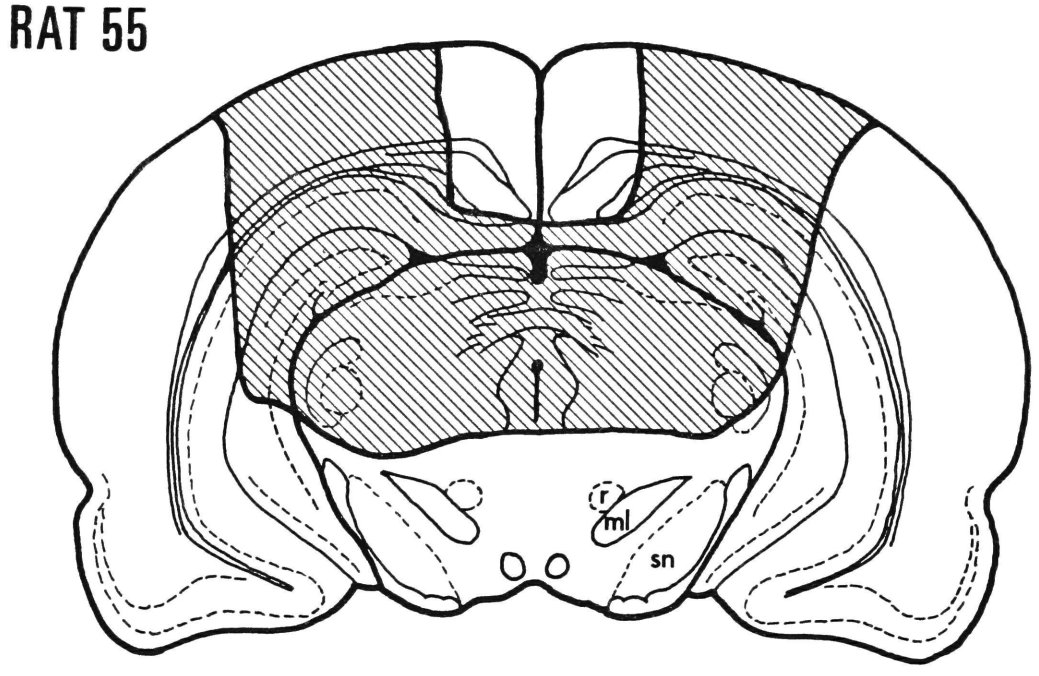

Figure 3-7. Coronal reconstruction of transection.

Conventions as in Figure 3-3.

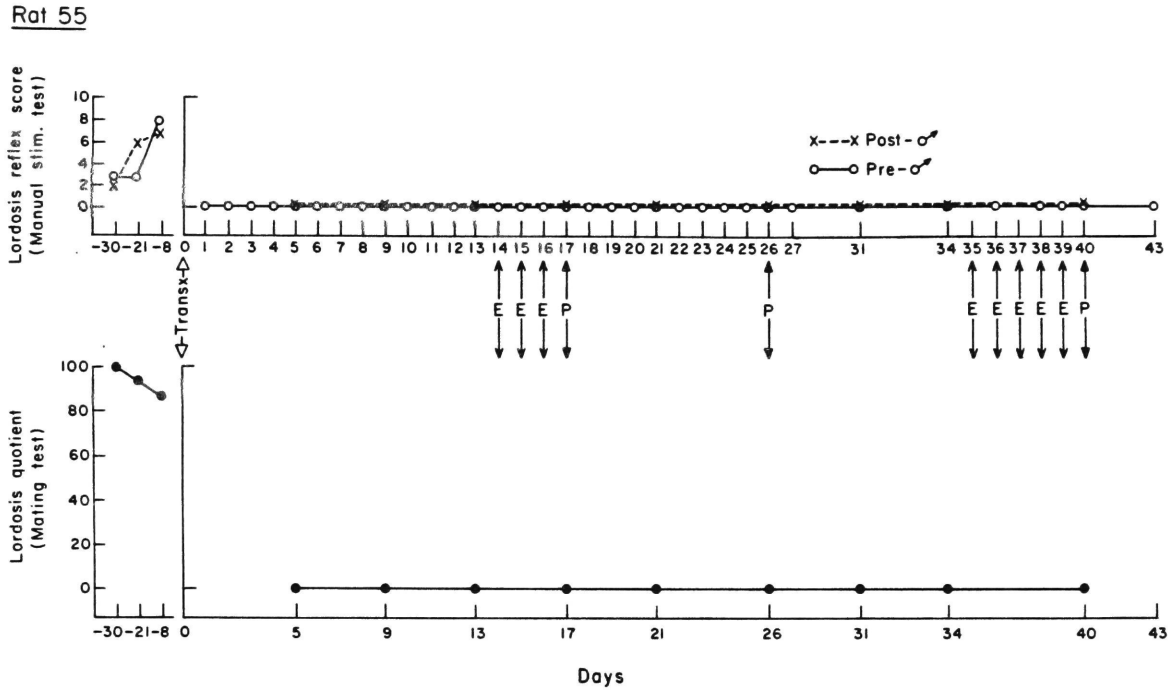

Figure 3-8. Lordosis test results. Plots show the history of lordosis test scores. Transection on day 0. Pre-operative scale collapsed with number of days preceding transection indicated below test value for that day. Post-operative scale linear in days. Arrows at "E" indicate subcutaneous injection of 10 μg. estradiol benzoate in 0.1 ml. sesame oil. Arrows at "P" indicate subcutaneous injection of 0.5 mg. progesterone in 0.1 ml. sesame oil. Arrows at "-E" indicate the day on which estradiol-containing silastic implant was removed, and lines connecting "-E" test scores become dotted.

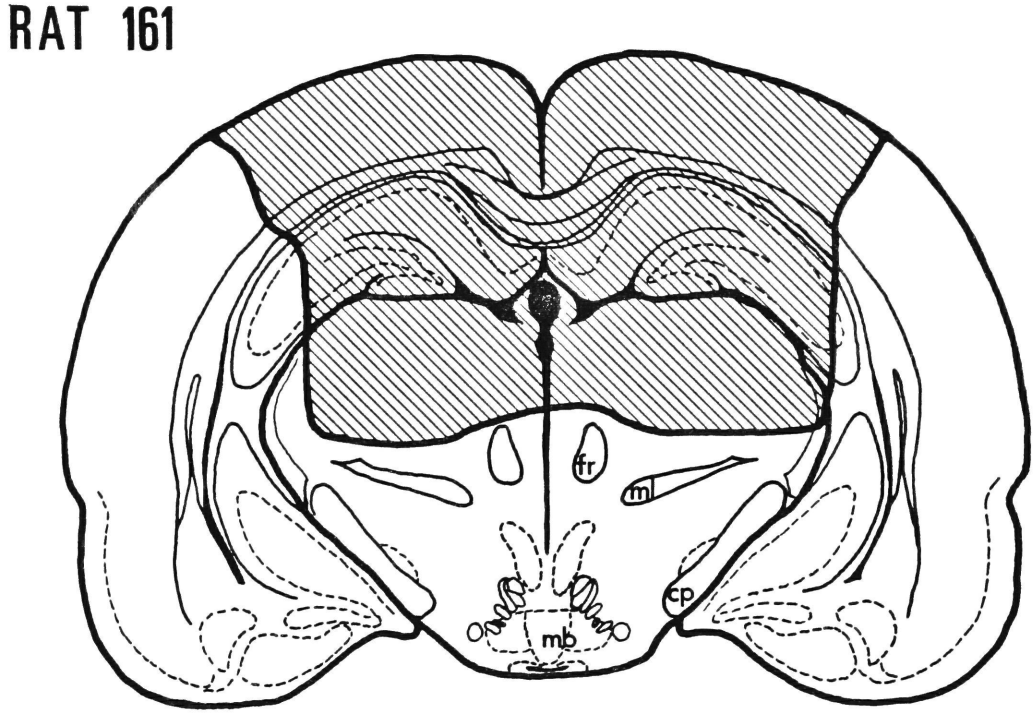

Figure 3-9. Coronal reconstruction of transection.

Conventions as in Figure 3-3.

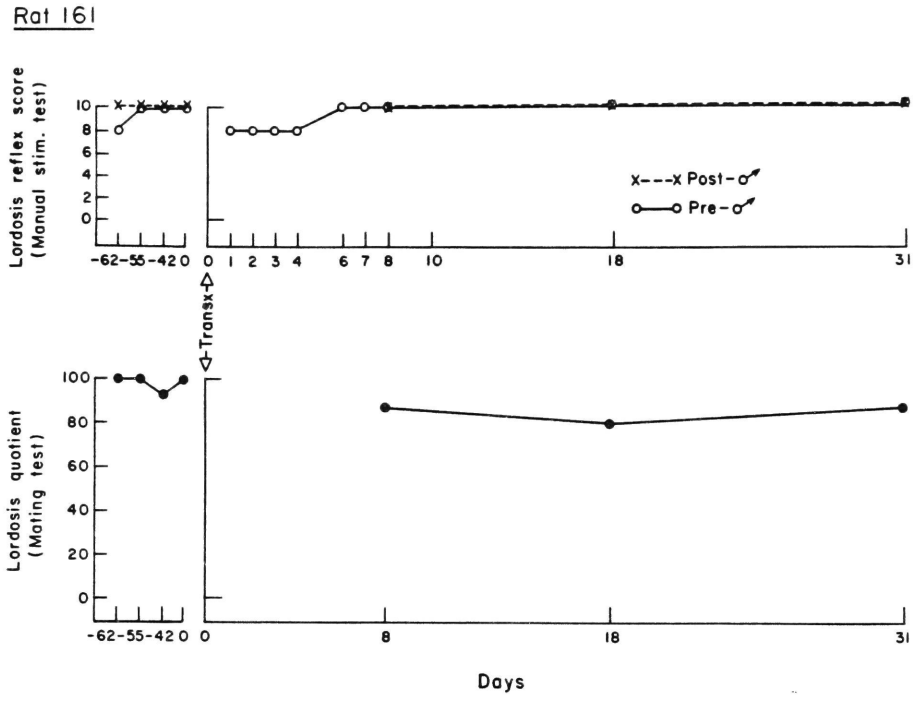

Figure 3-10. Lordosis test results. Conventions as in Figure 3-8.

Medial transections.

Eighteen animals received transections designed to selectively interrupt the medial pathway by which VMN fibers reach the lower brainstem. The extent and placement of transections in chronically surviving animals fell into three groups: A) Shallow medial transections (N = 2); B) Deep medial transection at or just anterior to the red nucleus (N = 5); and C) Deep medial transections posterior to the red nucleus (N = 2).

Shallow medial transections: Two animals received transections at the same rostrocaudal placement and depth as the group with dorsal-hemisections at the level of the red nucleus. These transections included the dorsal central grey at the level of the red nucleus, and extended to the bottom of the cerebral aqueduct (see Fig. 3-11 for comparison). Both animals were healthy and vigorous post-operatively, feeding and thermoregulation was normal, and no neurological or locomotory deficits were obvious. Both animals showed post-operative lordosis test results at pre-operative levels. When the estrogen-containing implants were removed and the animals were tested 2 weeks later, lordosis could not be elicited in manual or mating tests. A 9-day course of EB injections followed by P 6 hours before testing fully restored lordosis performance in male and manual tests.

Deep medial transections at and immediately anterior to the level of the red nucleus: The coronal reconstruction of the transection in rat 293 is shown in Fig. 3-11. This transection is typical of the width of transection in all animals in all medial groups. The transection extends slightly wider than the central grey and extends down into and nearly through the red nucleus bilaterally. This extent is representative of 2 of the other animals in this group. Two additional animals were included whose transections extended completely through the brainstem just rostral to the red nucleus.

Figure 3-12 shows the history of lordosis test results for rat 293, and this history is representative of each of these 5 animals' lordosis performance. Recovery of lordosis in manual tests was complete by day 2, and male rats reliably triggered lordosis in mating tests.

The estrogen implant was removed from rat 293 (see Fig. 3-12) and lordosis declined gradually day-by-day, until 8 days later, when neither mating nor manual stimulation could trigger the reflex.

Table 3-1 shows the mean pre- and post-operative lordosis performance by this deep medial group. Post-operative performance is nearly identical with pre-operative levels.

Rats with deep medial transections reaching the red nucleus were healthy and vigorous following transection, fed, thermoregulated and locomoted normally. These rats were allowed to survive for at least 2 weeks and no decline in post-operative performance was seen before

sacrifice. The two rats with complete medial transections rostral to the red nucleus failed to feed themselves, but otherwise appeared normal and vigorous. Despite intragastric feeding to maintain body weight, both rats died after only 1 male test, during the first week post-op.

Deep medial transections posterior to the red nucleus: Two animals (rats 296 and 297) survived chronically with medial transections of the same width (see Fig. 3-11) as previously described medial transections. These transections were located just posterior to the red nucleus, and extended to the bottom of the brain. Both of these animals failed to feed themselves for the first 3 weeks post-op, but maintained body weight during the last week post-op. Both rats locomoted only infrequently, had hunched-over postures, and other neurological deficits.

In contrast to slightly more rostral deep-medially transected animals, these rats showed reduced frequencies of responding in mating tests. The mean preoperative LQ of these animals was 96.5, mean post-operative LQ was 20. Like all medially transected animals, lordosis to manual stimulation was not affected by transection. The mean pre-operative post-male lordosis reflex score was 8.2, mean post-op. = 9.2 for these caudal, deep, medially transected animals. Supplemental EB+P treatment did not elevate the LQ, and the lordosis reflex score remained high.

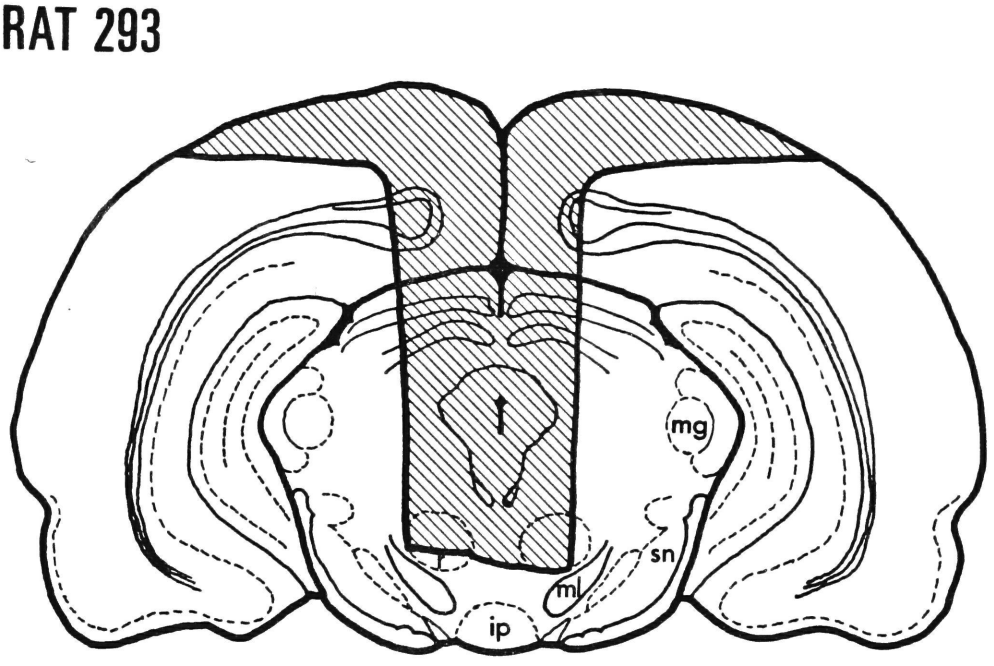

Figure 3-11. Coronal reconstruction of transection.

Conventions as in Figure 3-3.

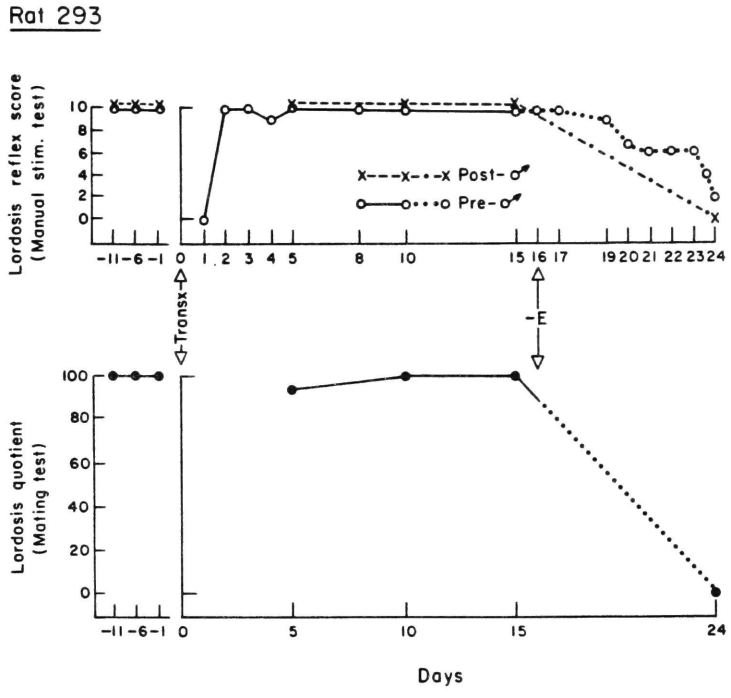

Figure 3-12. Lordosis test results.

Conventions as in Figure 3-8.

DEEP MEDIAL TRANSECTIONS (N = 5)

	Lordosis Quotient $\bar{X} \pm$ S.E.M.	Lordosis reflex score $\bar{X} \pm$ S.E.M.
Pre-op	99.5 ± 0.5	9.1 ± 0.5
Post-op	96.4 ± 1.8	9.4 ± 0.3

Table 3-1. Mean lordosis test scores.

Summary: Medial transections never interfered with the performance of lordosis to manual stimulation. Lordosis in mating tests was also elicited with pre-operative reliability, except when complete medial transections were made posterior to the red nucleus.

Parasagittal transections at the level of VMN.

Twenty-four animals were given bilateral parasagittal transections at the level of the VMN. These transections were designed to cut the lateral pathway as the fibers fan out from the ventromedial nucleus, before they turn caudally to descend in the lateral brainstem. Six animals survived for at least 3 weeks post-operative.

These parasagittally transected animals were healthy and vigorous following transection without obvious locomotory or neurological deficits. All fed and maintained body weight.

The most complete transection of the lateral pathway in this group was accomplished in rat 314 (Fig. 3-13). This histology was prepared in the coronal plane in order to accurately determine the extent of the transections. The transections are shown as heavy black lines, hatched areas surrounded by heavy black lines indicate ablated volumes, and unbounded hatching indicates degeneration. The top section shows the rostral-most level at which the transections are complete bilaterally, the middle section is at posterior VMN levels, and the bottom section shows the caudal-most level at which the transections are present bilaterally. The VMN is entirely contained within the rostrocaudal extent of these transections.

The history of lordosis test results for this animal is shown in Fig. 3-14. Lordosis could not be elicited in mating tests post-operatively. However, the lordosis reflex score recovered promptly following transection and is only slightly reduced during the post-operative period.

The extent of the transections in another parasagittally transected animal, rat 321, is shown in Fig. 3-15. This rat's lordosis performance was the least affected in the parasagittal group. The transection intercepts the course of many lateral sweeping fibers as they exit the VMN. However, these transections are only complete at mid-VMN levels and the transection on the right is not so deep as to cut all lateral-fanning VMN fibers.

The history of lordosis test results for rat 321 is shown in Fig. 3-16. Lordosis to manual stimulation recovered to preoperative levels promptly. By post-op day 10, lordosis was elicited with pre-operative reliability in the mating test.

The effect of transection on lordosis performance by the four other animals in this group was very similar. The transections were extensive enough to cut most of the VMN fibers fanning laterally as they exit the VMN. Furthermore, the lateral pathway was spared at a different location in each case. Performance of lordosis post-operatively was never severely or lastingly reduced in response to manual stimulation. Post-operative LQ's were reduced, the magnitude of this change varied from severe (as with 314) to nearly none (as with 321).

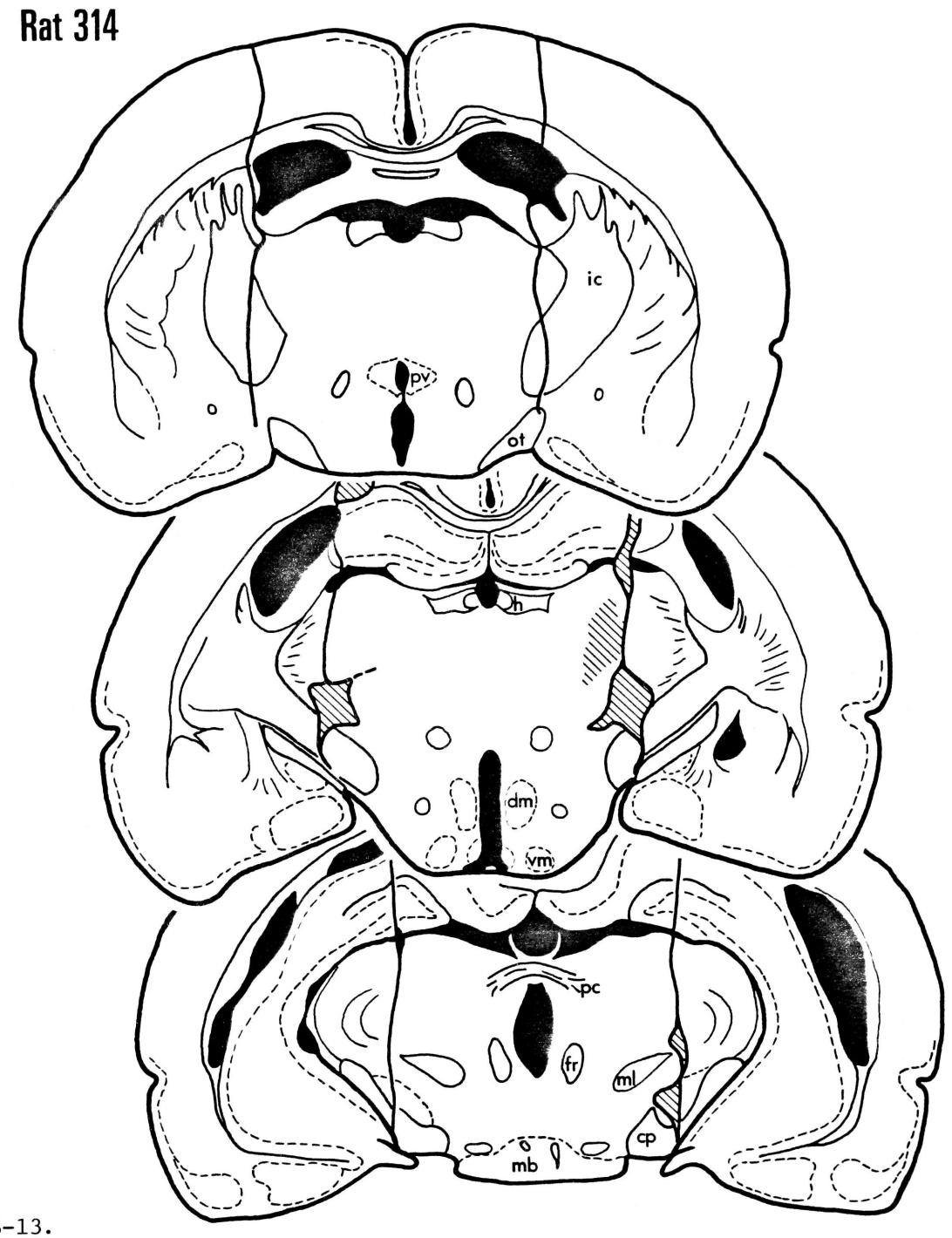

Figure 3-13.

Tracings of microprojections of coronal 100 μ-thick sections showing extent and placement of parasagittal transections.
Top section: rostralmost level at which transections were present bilaterally.
Middle section: posterior ventromedial nucleus level.
Bottom section: caudalmost level at which transections were present bilaterally
Conventions as in Figure 3-3.

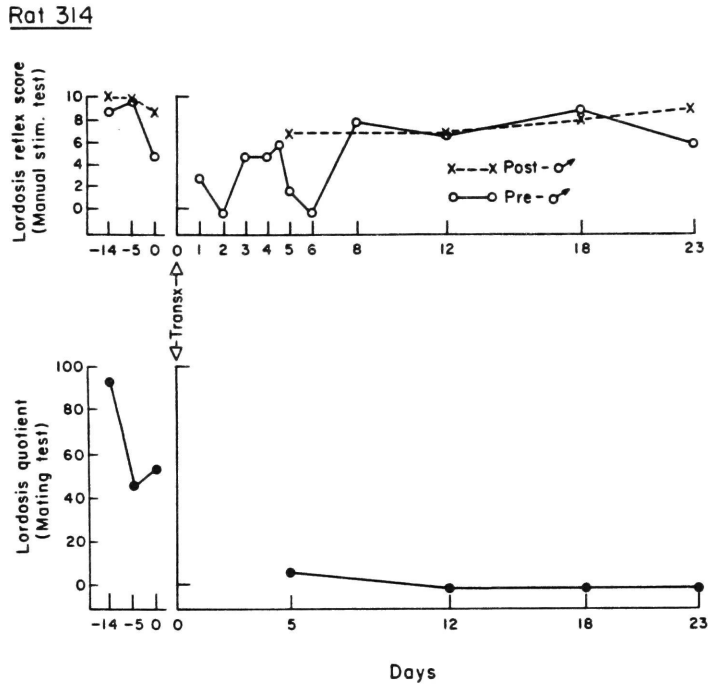

Figure 3-14. Lordosis test results. Conventions as in Figure 3-8.

Figure 3-15. Tracings of microprojections of 100μ-thick coronal sections showing extent and placement of parasagittal transections. Conventions as in Figures 3-13 and 3-3.

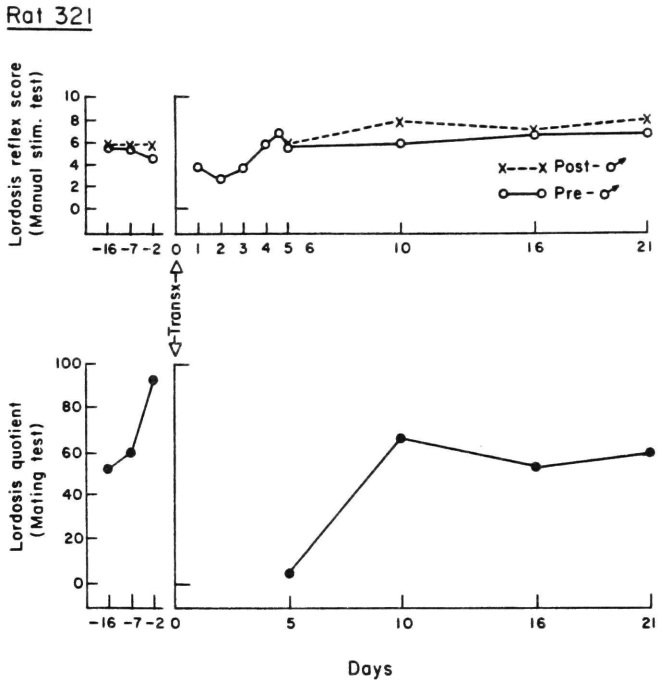

Figure 3-16. Lordosis test results. Conventions as in Figure 3-8.

Lateral transections.

Forty-six animals were given lateral transections of the brainstem in the coronal plane. These transections were designed to cut portions of the brainstem through which fibers from the VMN descend after they have fanned out from the VMN taking a lateral trajectory to lower brainstem levels. Twenty animals survived chronically, and these animals were healthy and vigorous following transection. They fed themselves, thermoregulated normally and did not show obvious neurological deficits.

Deep bilateral lateral transections: The results of two transected animals are presented in detail. These results are representative of a group of 11 animals with comparable bilateral lateral transections.

The coronal reconstruction of the transections in rat 272 is shown in Fig. 3-17. The transections are located in a coronal plane at the level of the posterior mammillary bodies. The history of lordosis test results for this animal is shown in Fig. 3-18. During the first month post-op, neither manual nor mating stimulation could elicit lordosis responses. When EB and P were administered in supplement of the estrogen replacement by implant, no improvement was seen in mating tests. However, fair lordosis responses could then be elicited manually. This result was typical of several animals in this group.

The coronal reconstruction of the transections in rat 259 is shown in Fig. 3-19. This animal's post-operative lordosis performance was the least affected of the deep bilateral lateral group.

These transections are at the same rostrocaudal level as rat 272, and are slightly wider dorsally. Note that the transection on the right side, however, is not as complete ventrally. The history of lordosis test results for this animal is shown in Fig. 3-20. In mating tests with estrogen replacement by implant alone, the LQ is reduced to 15% of the pre-operative level. In contrast, the lordosis reflex score is only slightly reduced and fully recovered later in the post-op period. In later mating tests with supplemental EB and P, rat 259 was able to show lordosis at nearly pre-operative levels. Supplemental estrogen alone, but not progesterone alone was effective in restoring the LQ in the mating test.

The mean lordosis performance supported by the implant alone in this deep bilateral lateral group is shown in Table 3-2. These transections reduced high preoperative LQ's to very low frequencies of response. Similarly, lordosis reflex scores indicating extreme lordosis to light contact were reduced to scores indicating minimal responses to strong stimulation.

Shallow bilateral transections: Two animals survived with much shallower bilateral lateral transections, limited to the upper half of the brainstem at its most lateral aspect. In both animals the transections are confined to the area of the medial geniculate and overlying tectum; in one animal at posterior mammillary levels, in the other animal at the level of the red nucleus. Performance of lordosis was equal to preoperative performance in both animals. Lordosis behavior was eliminated by removing the estrogen implant and fully restored by EB+P treatment in the absence of the implant.

Smaller bilateral or unilateral lateral transections: Histological reconstruction of the transections of four animals showed that their transections were 1) less deep and narrower than the deep bilateral lateral group or 2) unilateral. In the first case, lordosis performance in both mating and manual tests recovered to the pre-operative range by the end of the post-operative period. In the case of unilateral transection, no deficit was ever seen following transection.

Bilateral lateral transections at the ponto-mesencephalic junction: One animal survived with a deep bilateral lateral transection at the junction of the pons and mesencephalon, posterior to the deep bilateral lateral group. The coronal reconstruction of the extent and placement of these transections is shown in Fig. 3-21. The central grey was spared between the transections. The history of lordosis behavior by this animal is shown in Fig. 3-22. The post-operative lordosis reflex scores always fell within the pre-operative range. By the second mating test (day 12) the post-op LQ had recovered to pre-operative levels.

Summary: These bilateral lateral transections establish that transections of the lateral brainstem which interrupt the lateral descending VMN pathway at the level of the mammillary bodies reliably produce severe deficits in lordosis behavior. Smaller or unilateral transections do not have such dramatic or lasting effects. Supplemental EB and P could restore some degree of lordosis performance following lateral transections. When the effective transection strategy at mammillary levels was carried out at the pontine-mesencephalic junction, no lasting deficit was seen in lordosis behavior.

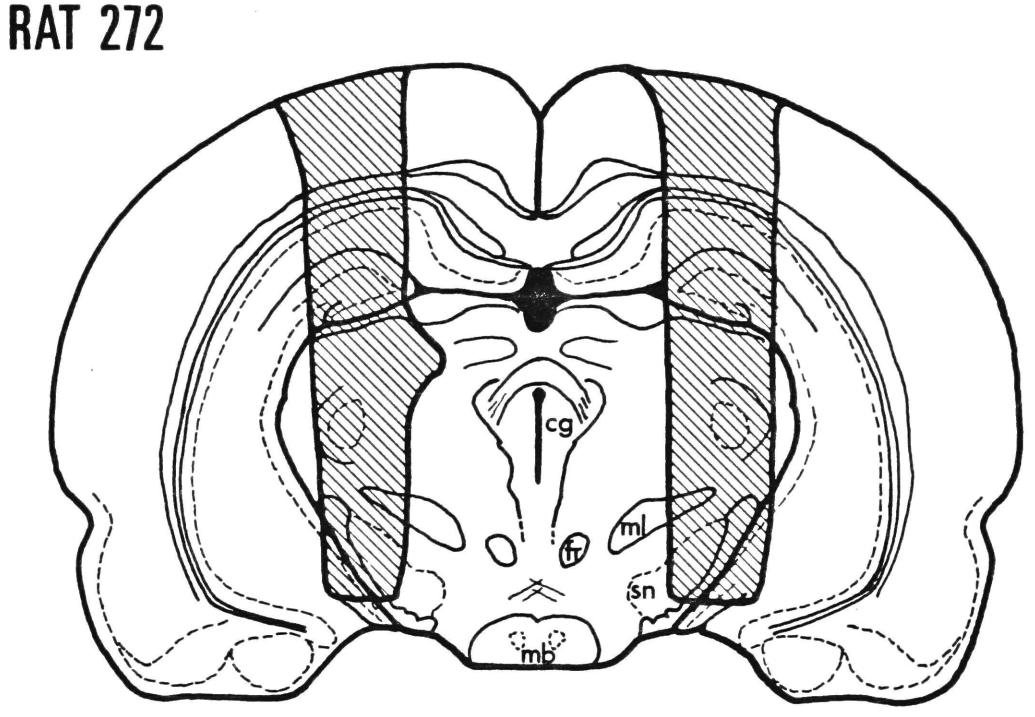

Figure 3-17. Coronal reconstruction of transections.

Conventions as in Figure 3-3.

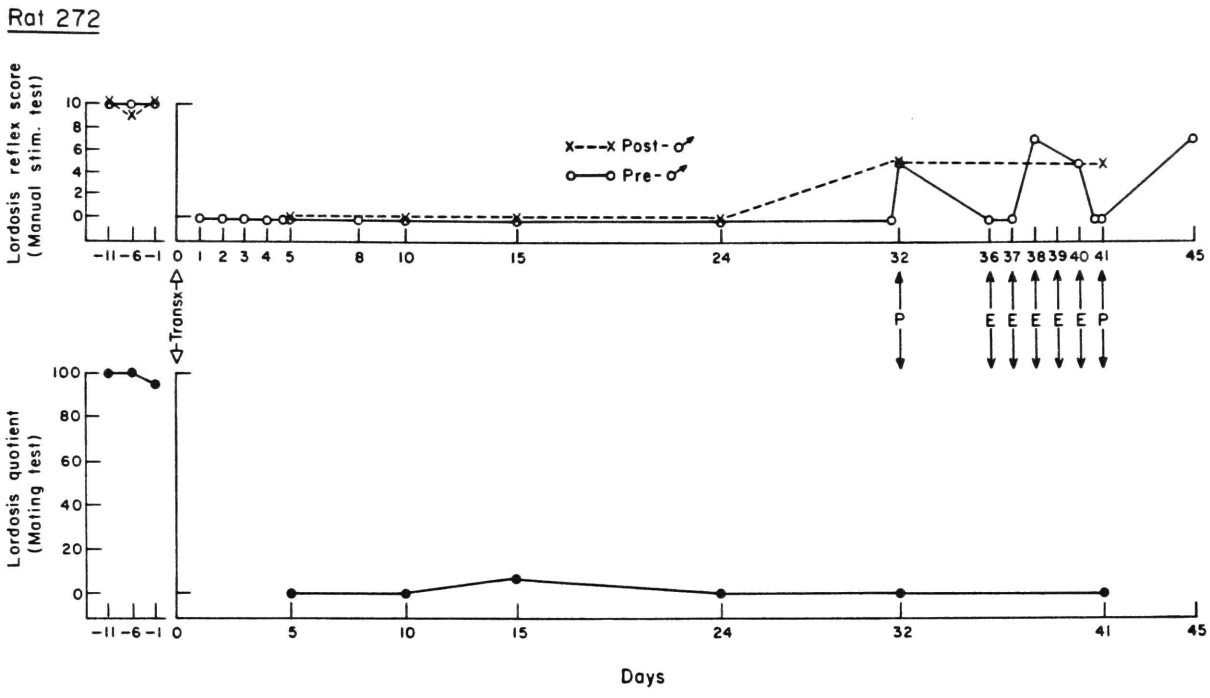

Figure 3-18. Lordosis test results. Conventions as in Figure 3-8.

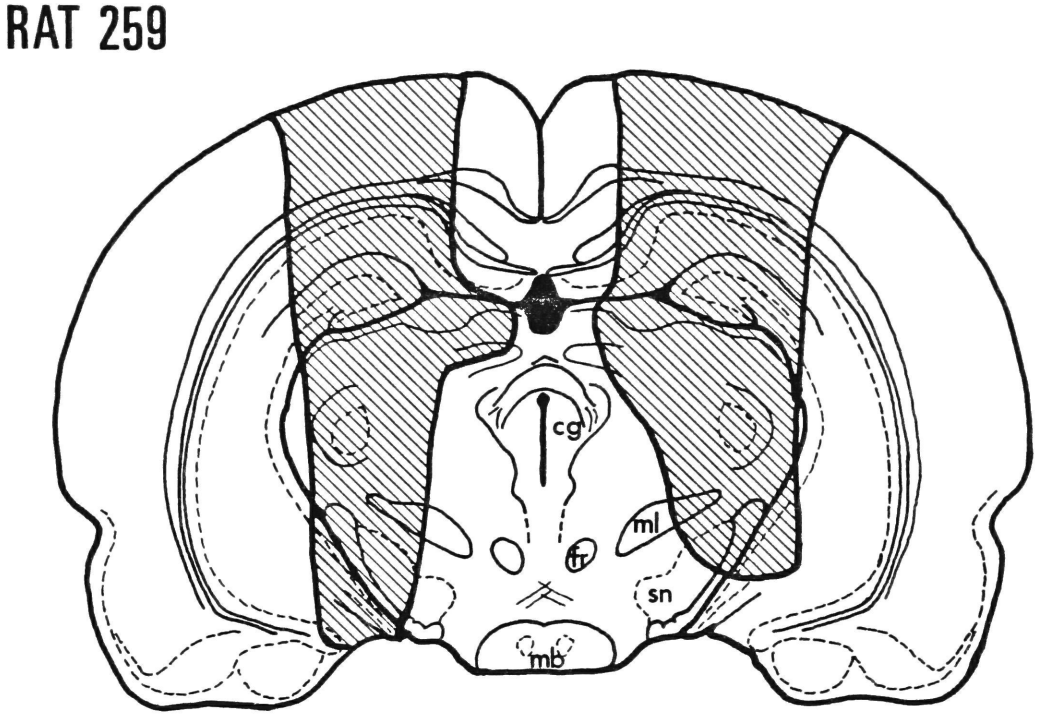

Figure 3-19. Coronal reconstruction of transections.

Conventions as in Figure 3-3.

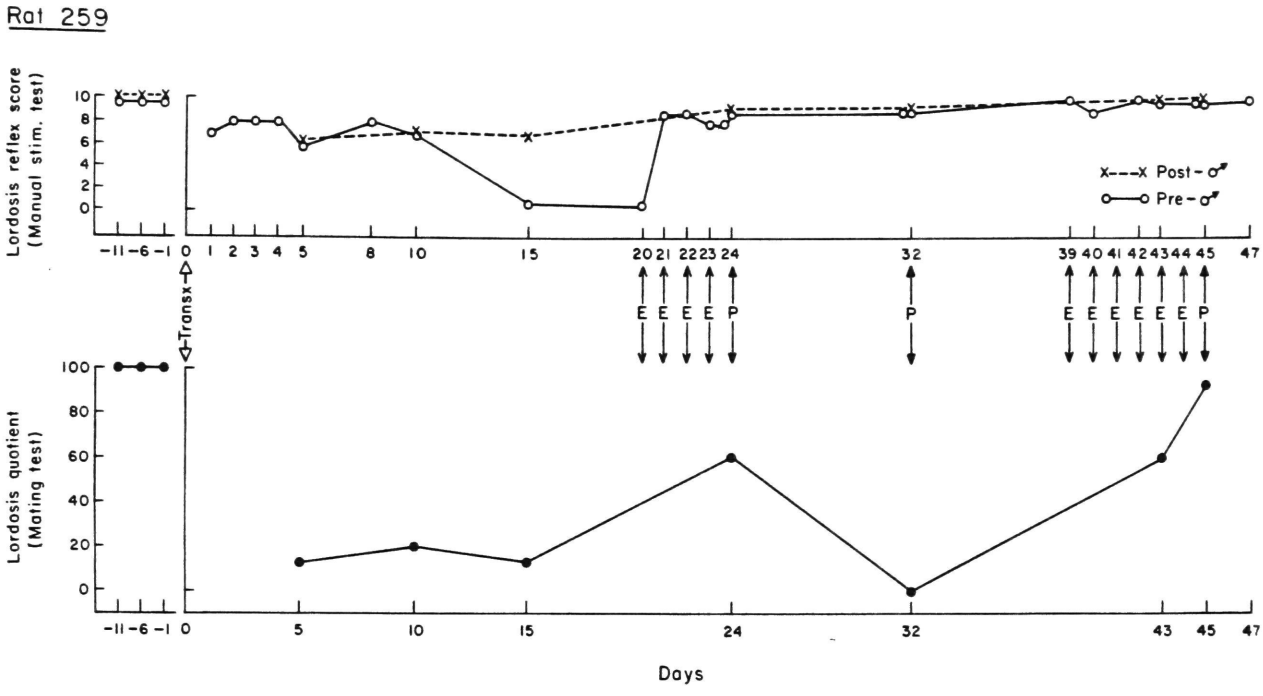

Figure 3-20. Lordosis test results. Conventions as in Figure 3-8.

DEEP BILATERAL LATERAL TRANSECTIONS (N = 11)

	Lordosis Quotient $\bar{X} \pm$ S.E.M.	Lordosis reflex score $\bar{X} \pm$ S.E.M.
Pre-op	98.3 ± 0.7	9.0 ± 0.4
Post-op	3.6 ± 1.2	3.2 ± 0.9

Table 3-2. Mean lordosis test results.

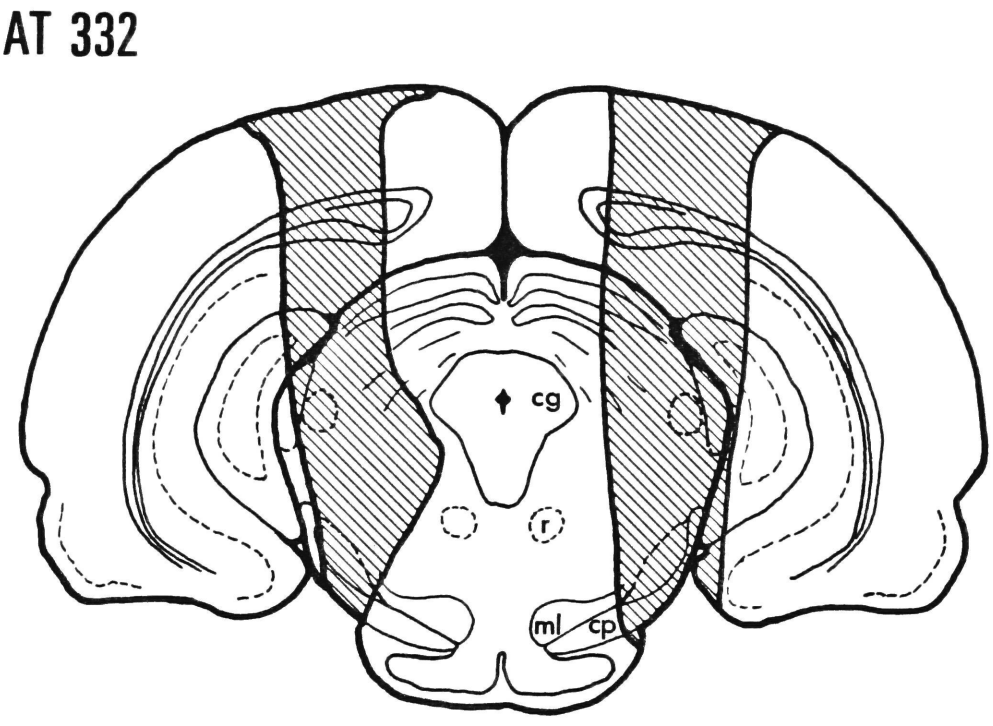

Figure 3-21. Coronal reconstruction of transections.

Conventions as in Figure 3-3.

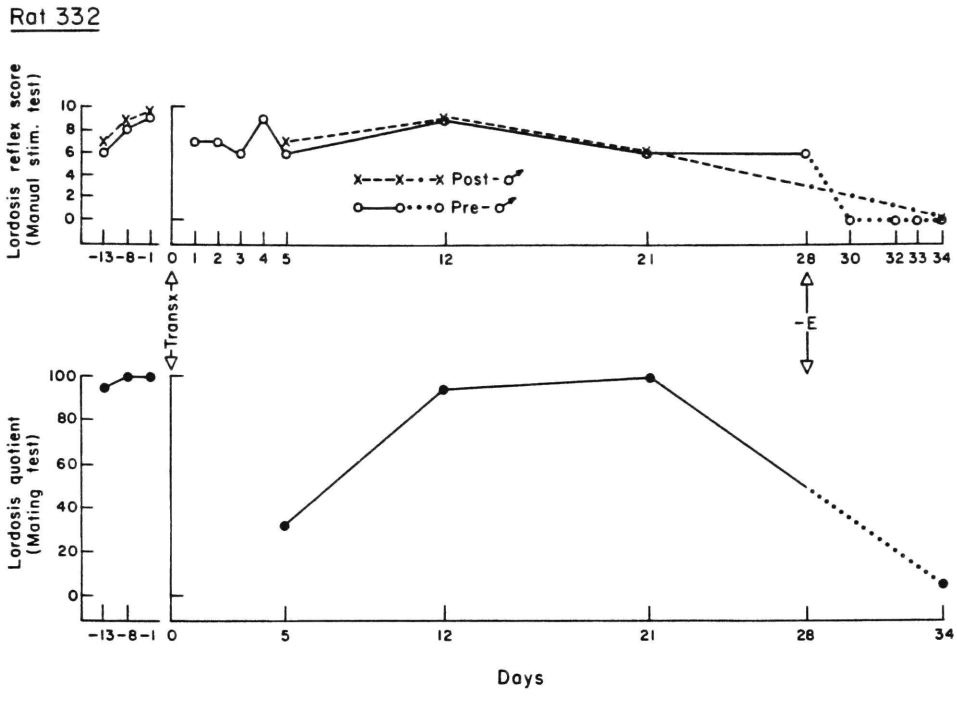

Figure 3-22. Lordosis test results.

Conventions as in Figure 3-8.

Rejection behaviors in mating tests.

In mating tests, lordosis may fail to occur if the female actively prevents the male from delivering copulatory mounts. Rejection behaviors during post-operative mating tests when LQ = 0 (lordosis did not occur) have been summed across all transection groups and the relative frequencies of responding in each non-lordosis category to all mounts and mount-attempts by the male are shown in Fig. 3-23. For comparison, the distribution of non-lordosis behavior by the same females during pre-operative tests is also shown. Following transection, there is not a systematic increase in the intense rejection categories, which prevent males from completing copulatory behavior.

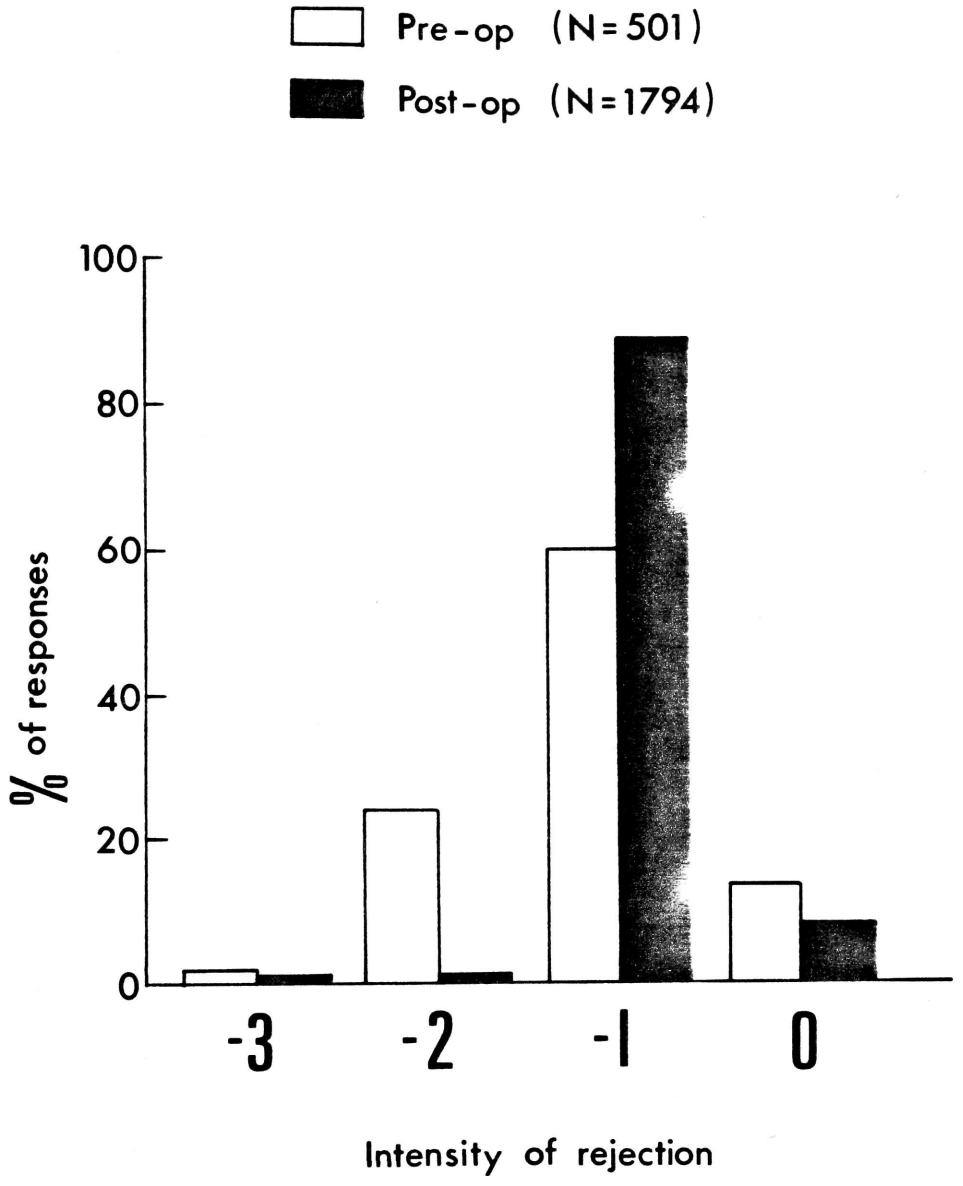

Figure 3-23. Percent of non-lordosis responses occuring in mating tests in each non-lordosis category. See Mating tests; lordosis quotients in METHODS for description of classes of rejection intensities.

DISCUSSION

The reductions in lordosis performance as a result of transection are not easily attributed to non-specific effects of surgery. Many severe (rat 161, Fig. 3-10) and limited (shallow medial, shallow bilateral lateral, unilateral lateral) transections did not interfere, even on post-op day 1, with lordosis performance. The stability of the lordosis reflex following unilateral lateral transections recalls the lack of an effect of unilateral "decerebration" reported by Kow, et al (1978). The elements of the supraspinal mechanism controlling lordosis at this rostrocaudal level appear completely refractory to unilateral transection, and to recover from non-specific surgical trauma promptly and completely.

All rats were able to stand and locomote post-operatively, even those which never showed lordosis following transection. When additional estrogen and progesterone was supplied to rats showing a partial reduction in LQ and lordosis reflex score, some improvement was usually seen in one or the other measures of lordosis (e.g. Figs. 3-18, 3-20). The ability of these animals to show occasional, well-integrated lordosis responses under favorable circumstances establishes their motor capacity to execute the response. Thus an absolute "motor incapacity" to perform lordosis following transection cannot account for the failure of lordosis to occur to previously adequate stimulation.

The failure of lordosis to occur in mating tests was also not due to a systematic increase in rejection behaviors which prevent the male from completing copulatory mounts and delivering adequate stimulation. Lordosis deficits here are better characterized as a simple failure to respond to vigorous stimulation.

Influence of forebrain cell groups (other than VMN) on lordosis.

No extra-hypothalamic areas of the forebrain have been shown to be required for lordosis. For example,the cerebral cortex is not necessary for the display of lordosis, nor for maintaining pregnancy (Davis, 1939). Indeed, surgical ablation has been seen to facilitate lordosis to stimuli which do not usually trigger the reflex and to potentiate the duration of lordosis in estrogen-progesterone treated females (Beach, 1944). This effect has been confirmed by functional decortication, or spreading depression, induced by application of potassium chloride or electrical stimulation to the surface of the cortex. KCl treatment (Clemens, et al., 1967) and electrical stimulation (Ross, et al., 1973) both facilitate lordosis in estrogen-replaced ovariectomized female rats. Olfactory bulbectomy increases the frequency of lordosis responses in mating tests of normal cycling and estrogen-progesterone replaced ovariectomized females, regardless of previous copulatory experience (Moss, 1971). This facilitation has also been demonstrated following replacement of estrogen alone, and is not attributable to peripheral anosmia (Edwards and Warner, 1972). Septal lesions also facilitate lordosis behavior in ovariectomized female rats treated with estrogen alone (Nance, Shryne and Gorski,1974).

Moreover, septal lesions increase the sensitivity of lordosis behavior to estrogen in ovariectomized females, allowing more lesioned than control females to respond following low doses of estrogen (Nance, Shryne, and Gorski, 1975).

Lesions of the amygdala (Nance, Shryne and Gorski, 1974) or hippocampus (Kimble, Rogers, and Hendrickson, 1967) have no effect on lordosis behavior. Furthermore, restricted lesions of the stria terminalis and medial corticohypothalamic tracts by which fibers project from these limbic structures strongly and directly to the hypothalamus do not affect estrogen-dependent lordosis responses to male rats (Brown-Grant and Raisman, 1972).

Lesions in the preoptic area (POA) reduce the amount of estrogen needed to support lordosis behavior in ovariectomized rats (Powers and Valenstein, 1972). Lisk (1962) and Lisk and Barfield (1975) report that small amounts of estradiol implanted in the POA reinstate lordosis in ovariectomized female rats. This effect has been interpreted as reflecting an estradiol-induced disinhibition of tonic POA inhibitory influences on more caudally located brainstem lordosis mechanisms. (Powers and Valenstein, 1972). In this regard, it is interesting that electrical stimulation of the POA during the first 6 hours of systemic estrogen treatment interferes with the lordosis responses of ovariectomized females tested two days later (Napoli, Powers, and Valenstein, 1972). Lesions of the medial forebrain bundle (MFB) through which POA fibers project to the lower brainstem (Conrad and Pfaff, 1976) do not interfere with lordosis (Hitt, et al., 1970; Rodgers and Law, 1967; Modianos, Delia, and Pfaff, 1976).

Lesions of the habenula and stria medularis thalami have been shown to interfere with feminine sexual behavior, increasing the number of minimal lordoses, decreasing soliciting behaviors and increasing avoidance of the male (Rodgers and Law, 1967; Modianos, Hitt, and Flexman, 1974). This habenular contribution may be responsible for the partial reductions in lordosis quotients seen to follow dorsal hemisections in the posterior thalamus, but cannot account for the more pronounced lordosis reflex deficits with more caudal hemisections, which spared the habenula and fasciculus retroflexus.

Medially placed lesions in the AHA have been shown to prevent lordosis responses to copulating males (Law and Meagher,1958; Singer, 1968). Singer (1968) reports that the failure to show lordosis by such AHA-lesioned females is characterized by vigorous resistance by the females to any attempt on the part of males to mount them. The role of the AHA in the control of the lordosis reflex therefore, is not clear. The lordosis deficit due to AHA lesions may only reflect increases in rejection behaviors effective in preventing males from providing adequate copulatory stimulation. The adequacy of such stimulation, if provided, to elicit lordosis responses following AHA lesions must still be tested. In this regard, the medial pathway by which AHA fibers reach the lower brainstem (Conrad and Pfaff, 1976) was not shown to be necessary for lordosis - its interruption by deep, medial transections did not reduce lordosis performance.

Thus,the lordosis deficits seen following various mesencephalic transections are not solely attributable to the interruption of fibers

descending through the midbrain from forebrain areas other than the ventromedial nucleus of the hypothalamus. Dorsal hemi-sections of the brainstem which interrupt most or all of the descending VMN fibers at the level of the red nucleus reduce or eliminate lordosis behavior in estrogen-primed female rats. These considerations (see also INTRODUCTION) indicate not only that descending VMN pathways to the lower brainstem mediate estrogen-dependent lordosis behavior, but also that other forebrain cell-groups, whose connections with the lower brainstem are completed ventral to these transections, cannot fully support lordosis behavior by themselves.

Influence of fibers ascending through mesencephalic levels in the control of lordosis.

Kow, Montgomery and Pfaff (1977) proved that a supraspinal mechanism must be involved in the control of lordosis by showing that complete spinal cord transections at thoracic levels abolish the lordosis reflex. By placing partial transections at this level, Kow, et al., (1977) also established that the fibers necessary and sufficient for lordosis run in the anterolateral columns of the spinal cord. The rest of the spinal cord (ventromedial columns and dorsal half of cord) can be transected and lordosis can still be performed. Bilateral transections including the anterolateral columns, however, caused severe deficits in the strength of the reflex and the reliability of its elicitation, sometimes eliminating the behavior. Although these spinal transections do not prove that an ascending supraspinal limb is required for lordosis, such may be the case. The course of fibers ascending from the anterolateral columns has been determined (Zemlan, Leonard ,

Kow and Pfaff, 1978). Figure 3-24 shows the course of degenerating anterolateral column axons through medullary, pontine, mesencephalic and thalamic levels. Transections which interrupt these ascending projections at thalamic levels (e.g. rat 161, fig. 3-9) did not have severe effects on lordosis. These ascending projections were also cut in the effective bilateral lateral transections. However, bilateral lateral transections at the pontine - mesencephalic junction (see rat 332, Fig. 3-21) also interrupt these spinothalamic fibers originating in the anterolateral columns (sparing VMN axons), yet produce no deficit in lordosis (see Fig. 3-22). Therefore, the lordosis deficits following mesencephalic transections cannot be attributed solely to spinothalamic or spinotectal fibers which may contribute to an ascending limb of a supraspinal mechanism controlling lordosis.

The participation of inputs to the VMN must also be considered. Lesions which destroy the dorsal half of the central grey (CG) and the adjacent subtectum produce an immediate decline in lordosis performance (Sakuma and Pfaff, 1979). Previous reports (Chi, 1970) have not demonstrated degeneration within the VMN following lesions in and around the central grey. However, recent work utilizing the ^{3}H-amino acid technique does show an ascending projection from the central grey to the VMN (Krieger and Pfaff, pers. comm.). Based on this preliminary work, however, this projection reaches the hypothalamus along a medial trajectory. This pathway was completely interrupted by anterior deep medial transections, and these transections resulted in no deficit in lordosis. A necessary role in the control of lordosis by these fibers

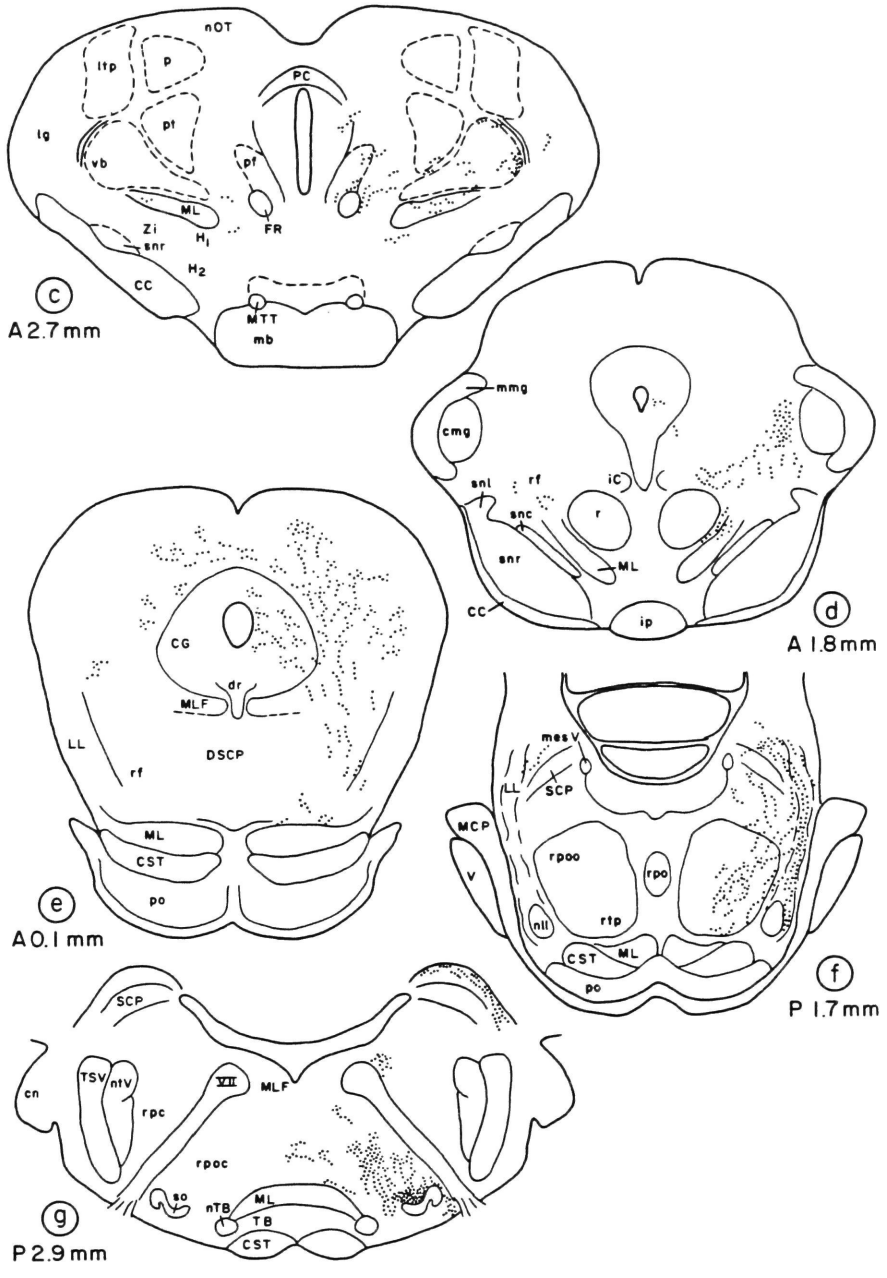

Figure 3-24. Course of degenerating (dots) anterolateral column axons following transection of anterolateral column in spinal cord. From: Zemlan, Leonard, Kow and Pfaff, 1978.

then, appears unlikely. It should also be noted that the effects of VMN and CG lesions show a different time course -- CG lesions reduce lordosis performance immediately (Sakuma and Pfaff, 1979b) whereas VMN lesion effects are maximal only after 12 - 60 hours (Pfaff and Sakuma, 1979b). Similarly, CG stimulation produces an immediate facilitation of lordosis (Sakuma and Pfaff, 1979a) whereas lordosis is facilitated after 15 - 60 minutes of stimulation at VMN sites (Pfaff and Sakuma, 1979a). The time course of these effects indicates that while cells in and around the central grey may participate in the control of lordosis on an immediate or mount-by-mount basis, VMN cells probably do not. A mount-by-mount participation of the VMN in the control of lordosis also appears unlikely on analogous neurophysiological grounds. Bueno and Pfaff (1976) have recorded single units in the VMN of urethane anesthetized, ovariectomized female rats. Estrogen treatment tends to increase the number of recordable cells, but this effect is primarily due to an increase in the number of cells with low (less than 4 per second) resting discharge rates. Furthermore, very few hypothalamic cells respond to cutaneous stimulation relevant to lordosis, and those responses following cutaneous stimulation are sluggish. Thus, it does not seem likely that sensory input to these VMN cells participates in triggering the lordosis reflex, which shows a latency of about 160 msec. to cutaneous stimulation by the male (Pfaff and Lewis, 1974).

Finally, even if ascending neural input to the VMN were critical to the control of lordosis, it would then be obligated to manifest any such involvement over VMN efferent fibers. Thus, from the con-

siderations above it seems that mesencephalic transection effects cannot be attributed solely to interruption of VMN input ascending from the midbrain, but must include the effects of VMN output to the lower brainstem.

Contribution of medially vs. laterally descending VMN axons in the control of lordosis.

The elimination of lordosis following mesencephalic transections cannot be attributed solely to the individual interruption of either the medial or the lateral pathway by which VMN fibers reach the lower brainstem. Medial transections never reduced lordosis performance if perineal stimulation was supplied. Only when medial transections were located posterior to the red nucleus were reductions in the LQ shown, and at this posterior level, unrelated neurological deficits may have interfered with mating. Parasagittal transections, which interrupt most of the fibers fanning outward from the VMN to reach a lateral descending trajectory, did not eliminate lordosis responses. Thus the lateral pathway need not be intact for lordosis to occur, and all of the lateral fibers are not necessary for lordosis if the medial pathway is intact. Among all of the parasagittally transected animals, no single subset of fibers in the lateral pathway could be shown to have escaped transection in every animal. Therefore, a critical role in the control of estrogen-dependent lordosis cannot be attributed to any particular subset of laterally descending fibers.

Thus, when the medial pathway is cut, the lateral fibers alone can support lordosis. Similarly, when lateral pathways are transected, the medial pathway and escaping lateral fibers allow lordosis to occur. Yet, the control of lordosis does not seem to be shared equally by VMN fibers running in the lateral vs. the medial pathway, because bilateral lateral transections significantly reduced lordosis performance, and medial transections do not. In the absence of the medial pathway, the lateral fibers support lordosis when females are treated with estrogen alone. When the lateral brainstem is transected bilaterally, however, the medial pathway and escaping lateral fibers were not able to support preoperative levels of lordosis intensity and frequency. A deficit sometimes persisted even with supplemental EB or P. Fibers in the lateral brainstem may normally contribute more to the control of estrogen-dependent lordosis than their medially descending counterparts. This assymetry may be attributable to functional differences or to differences in the number of VMN fibers descending in the respective pathways (i.e. "pro-rata lordosis reductions according to the number of VMN axons cut).

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