

directed a three- to fourfold increase in the incorporation of [<sup>3</sup>H]leucine as compared to the value obtained in the absence of mRNA. Treating the RNA with formaldehyde to reduce its ordered structure or, briefly, with mild alkali to partially degrade the mRNA resulted, respectively, in a slight loss and a slight enhancement of activity. In contrast, a much smaller amount of rabbit reticulocyte polysomal RNA (6  $\mu$ g) induces a greater than 30-fold increase in the incorporation of [<sup>3</sup>H]leucine. Rabbit globin and Q $\beta$  coat protein contain comparable amounts of leucine.

Although it is evident that the phage RNA is a less efficient template for protein synthesis in this cell-free system, the product, as analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, consists of a single polypeptide which comigrates with authentic Q $\beta$  coat protein (Fig. 1). The synthesized product yields tryptic peptides that correspond to each of the major Q $\beta$  coat protein tryptic peptides (Fig. 2), and also yields one prominent peak containing alanine (Fig. 2A, fractions 109–114) and two smaller peaks (52–53, 55–56) that are absent or present to only a small extent in the coat protein. These extra peptides may be related to the processing of an initial, Q $\beta$  coat peptide (fMet-Ala-; that is, formylmethionylalanine) or possibly to the partial translation of one of the noncoat cistrons. It is apparent, however, that, as in bacterial cell-free systems, the Q $\beta$  coat protein is translated *in vitro* in marked preference to the other two Q $\beta$  cistrons (8).

The fact that the Q $\beta$  coat cistron is translated in a mammalian cell-free system emphasizes the universality of the translation process. At the same time, it does not rule out subtle translational discriminatory mechanisms that may operate *in vivo*, but which are overcome by selecting conditions favorable for the translation of a specific, heterologous mRNA. This observation does have rather important practical implications, however, because it provides an operational connection between a mammalian cell-free system and a bacterial virus mRNA in which a variety of suppressible termination mutants are available. Thus, the system, in combination with an appropriate amber mutation in the Q $\beta$  coat protein, should provide a technique for surveying tRNA's from mutagenized lines of mammalian cells for their ability to suppress amber mutations. Recent ex-

Table 1. Protein synthesis in response to bacteriophage Q $\beta$  mRNA. Each reaction mixture contained the components, and was incubated under conditions described in the legend to Fig. 1, except that [<sup>3</sup>H]leucine plus 19 unlabeled amino acids were used. Where indicated, 6  $\mu$ g of rabbit reticulocyte polysomal RNA was added. The reaction mixtures were precipitated with trichloroacetic acid, and the precipitate was then assayed. The modified Q $\beta$  RNA's were incubated in 0.01M tris, pH 7.2, containing either 3 percent formaldehyde or in 0.01M tris, pH 9.0, at 37°C for 30 minutes and dialyzed against H<sub>2</sub>O.

Addition	[ <sup>3</sup> H]Leucine incorporated (pmole)
None	0.24
Q $\beta$ RNA	0.87
Q $\beta$ RNA, formaldehyde treated	0.56
Q $\beta$ RNA, pH 9.0 treated	1.27
Rabbit reticulocyte polysomal RNA	8.69

periments involving the use of Q $\beta$  and R17 bacteriophage mRNA's in a variety of mammalian cell-free systems suggest that this approach will be generally applicable (10). The identification of a suppressor-containing mammalian cell line would be of obvious value in studies involving animal viruses and cultured cells.

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## Genital Sensory Field: Enlargement by Estrogen Treatment in Female Rats

**Abstract.** *Recordings of neuronal activity in the pudendal, genitofemoral, and pelvic nerves indicate that the sensory fields of these three nerves are the perineum, the caudal abdomen, and the vagino-cervical area and rectum, respectively. The sensory field of the pudendal nerve was significantly larger in estrogen-treated ovariectomized female rats than in uninjected controls. This effect of estrogen was not mediated by pudendal efferents.*

Recent analyses of hormonal influences on behavior and the nervous system have emphasized the effects of hormones directly on the brain (1) and spinal cord (2). However, hormones may also influence behavior by altering peripheral sensory mechanisms. In humans, acuity of the senses of touch, taste, and olfaction are altered by hormones (3), and on the basis of behavioral responses in animals, it has been proposed that hormones may act at the periphery to alter sensory input

(4). However, there appears to be no previous neurophysiological evidence that hormones change peripheral neural activity. In one attempt to demonstrate an androgenic influence on penile sensitivity, recordings from the pudendal nerve in cats revealed no significant effect of androgen (5).

Perineal stimulation in female rats occurs prior to intromission, during copulatory thrusting by the male. This stimulation is likely to play a major role in facilitating the mating stance

in rats, the lordosis reflex, which is estrogen-dependent. In the present study, we investigated the innervation of the sensory field of the perineal region in female rats and the way in which the size of this field is influenced by systemically administered estrogen. Since vagino-cervical stimulation facilitates lordosis (6), initiates the secretion of progesterone necessary for pregnancy (7), and can facilitate or inhibit sperm transport from the vagina into the uterus (8), another purpose of our study was to map the innervation of the vagina and cervix.

The sensory field of the pudendal nerve was determined by recording from axonal populations in the nerve with silver hook electrodes [0.006 inch in diameter (Fig. 1C)], while mechanical stimulation was applied to the fur and skin (9). Stimuli were applied by brushing small tufts of fur briskly or scratching the skin firmly with a dissecting needle.

In order to establish reliability of

the recordings, a second placement of the electrodes was made several millimeters farther caudally (that is, peripherally), after the field was established with the first placement; the field was then replotted. After the replotted with the electrodes in the second position, the pudendal nerve was severed between the recording electrodes and the spinal cord; the field was then plotted a third time, thus eliminating possible centrifugal effects mediated by the pudendal nerve.

Sprague-Dawley female rats obtained from Camm Research Laboratories, Wayne, New Jersey, were ovariectomized 3 to 9 weeks prior to testing. Twelve micrograms of estradiol benzoate were injected subcutaneously in the scapular region every other day for 1 to 4 weeks immediately prior to testing. Only those females were used which displayed an "excellent" (6) lordosis in response to palpation of the flanks and perineum immediately prior to testing. The control group received

no injections, and none showed any indications of lordosis in response to this palpation. The rats were anesthetized with Urethane (Merck, 20 percent solution) at a dosage of 1.6 g/kg.

The sensory field of the pudendal nerve extends from the base of the clitoral sheath to the base of the tail in the midline and laterally along the inner surface of the thigh (Fig. 1A). Responses were also elicited from the posterior outer surface of the thigh. Careful mapping of the ventral field indicated, in almost all cases, a strictly ipsilateral field (11); a sharp boundary of responsiveness occurred at the midline even in the case of the clitoral sheath.

Except for the clitoral region (which is covered by short, fine abdominal fur), the abdominal sensory field of the pudendal nerve coincided closely with a triangular patch of long, coarse yellowish fur (12). This patch of fur is continuous with the long, coarse yellowish fur of the dorsal body surface. There is a distinct boundary running across the lower abdomen, where the coarse fur ends and the fine fur of the abdomen begins; the sensory field of the pudendal nerve rarely extended into this short fur region. We never observed "holes" in the field; that is, whenever a response was elicited near the field border, it was also elicited closer to the center of the field.

The major effect of the estrogen treatment was to expand the sensory field toward the borders of the patch of long fur. In females not receiving the hormone, the responsive area was restricted to the central portion away from the borders (Table 1). The following components of the sensory field of the pudendal nerve were significantly larger in the ovariectomized, estrogen-treated condition than in the ovariectomized, untreated condition: (i) the length of the clitoral field (the maximum distance at which a response could be obtained from the tip of the clitoral sheath up the abdomen); (ii) the area of the entire responsive region; (iii) the length of the perineal-thigh component (maximum anterior-posterior extent); (iv) the maximum lateral distance from the midline at which a response could be obtained (perineal-thigh field width). Responses at the midline occurred in 8 of the 16 estrogen-treated rats but only in 3 of the 18 rats in the control group, a significant difference [ $P = .048$ , Fisher test (10)].

The effect of hormone injections on the size of the sensory field was great

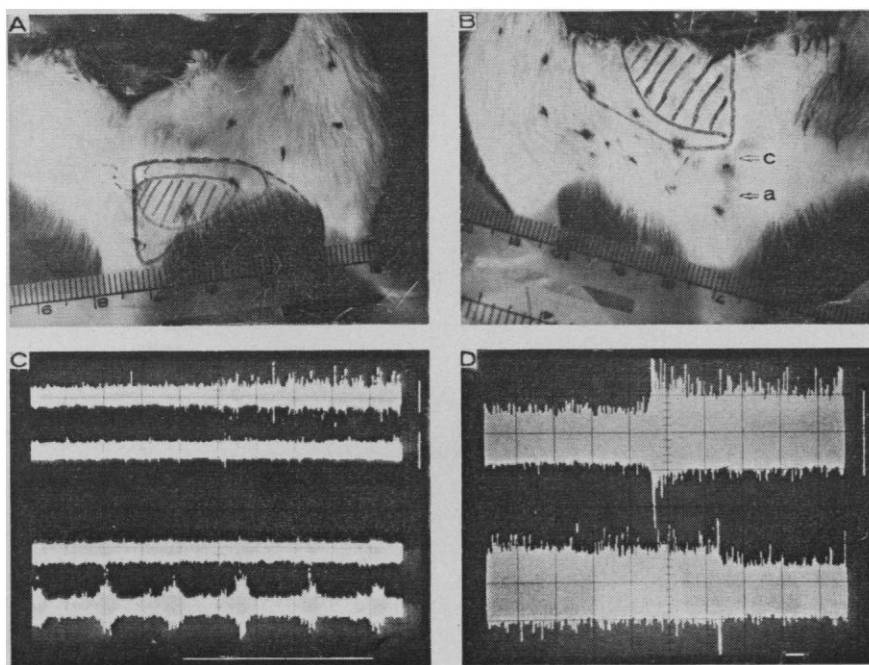


Fig. 1. (A) Sensory field of the left pudendal nerve, estrogen-treated rat. The solid line is the border of the responsive area, and the striped inner region represents the area of relatively strong responses. In this individual, this inner region is somewhat larger than usual. The dotted line delimits the border of the long, yellowish fur and the sensory field extends beyond this region only at the clitoral sheath. (B) Sensory field of the right genitofemoral nerve. The solid outer line is the border of the responsive area, and the striped inner region indicates a region of relatively strong responses. Note that the field extends to the base of the clitoral sheath (c) in the midline. (a) anus. (C) Specificity of perineal (pe) versus pudendal (pu) sensory fields. In 1 the vaginal cervix was stimulated after the middle of the trace, as described in the text. Note the absence of response concurrently in the pudendal nerve. In 2 the perineal fur was brushed six times. Note the absence of response concurrently in the pelvic nerve. Calibration in (C) and (D): time, 1 sec; voltage, 40  $\mu$ V. (D) Persistent response of the right genitofemoral nerve to pressure maintained on the abdominal region outlined in (B). Gentle pressure was applied just before the middle of the top trace and was released after the middle of the lower trace. The two traces are continuous. Note that the response was waning, but persisted for more than 20 seconds.

enough to permit subjective differentiation between estrogen-treated and non-estrogen treated females. At the end of each day's work, after the fields were plotted but before the rats' uteri were weighed or the injection history was consulted, we made a judgment as to the hormonal condition of the female. We correctly identified nonestrogen-treated females in 13 of 18 cases and estrogen-primed females in 12 of 15 cases [ $P < .025$ , Fisher test (10)].

There was an increase in responsiveness as the stimulus was moved from the periphery to the center of the field. Both the number of units and the amplitude of the spikes increased. A similar increase in sensitivity in the center of the field has been reported in the genital sensory field of the male cat (5). The more sensitive region in the female rats formed a semicircle of approximately 1 cm diameter with its center about halfway between the clitoris and anus (Fig. 1A). There were no obvious morphological characteristics which corresponded to this more sensitive region.

In the sensory field, mechanical stimulation of the skin or deflection of the fur, as little as a single hair, elicited a rapidly adapting response. The response to clitoral sheath brushing was characterized by spikes which were lower in amplitude and had a faster rise time than the units that responded to stimulation of the long fur.

We determined the reliability of the size and shape of individual sensory fields by placing the electrode pair into the pudendal nerve in a second position, closer to the periphery. For the combined estrogen and control groups, the correlation coefficients were significant for each measure (i to iv above) compared on the first and second placements [Spearman rho between .76 and .85;  $P < .005$ , one-tailed,  $n = 21$ , in each case (10)]. Thus, by recording the activity of a population of axons, we obtained a representative sample of the size of the entire sensory field. Since the electrodes were placed randomly within the nerve, and one part of the nerve contained fibers representing essentially the same field as a different part of the nerve, the field representation appears to be rather homogeneously distributed with the nerve.

To test for centrifugal effects mediated by the pudendal nerve, this nerve was cut proximal to the recording electrodes following the second electrode placement. For each field component (i to iv above), there was a significant

Table 1. Effect of estrogen on the genital-sensory field. Dimensions given are the medians; interquartile ranges are in parentheses. The ratios of differences [(injected — noninjected) as percentages are as follows: clitoris field length, + 75.0; perineum and thigh field width, + 26.3, length, + 22.0; area, + 31.9. Calculated on median values.

Number of animals	Field of clitoris (length, mm)	Field of perineum and thigh		
		Width (mm)	Length (mm)	Area (mm <sup>2</sup> )
18	4.0 (0–7.0)	<i>Ovariectomized</i>		
		19.0 (15.0–23.0)	16.8 (13.5–23.0)	210 (114–330)
16	7.0 (6.1–9.8)*	<i>Ovariectomized + estrogen</i>		
		24.0 (21.3–27.3)*	20.5 (17.3–23.3)†	277 (245–369)†

\*  $P < .01$ ; †  $P < .05$ ; Mann-Whitney U-test, one-tailed (14).

before-after transection correlation [Spearman rho between .60 and .87;  $P < .05$ , one-tailed,  $n = 10$  in each case (10)]. Since the shape and dimensions of the sensory fields of the pudendal nerve were not significantly altered by transection of the nerve, the effect of estrogen treatment is apparently not mediated by centrifugal influences in this nerve, although we cannot exclude the possibility of centrifugal influences mediated by other nerves innervating the same region.

The pelvic nerve, which lies adjacent to the pudendal nerve, was activated by stimulation of the vaginal wall, rectal wall, or cervix. Stimulation of the cervix was distinguished from stretching the vaginal wall by first exerting a force against the cervix with a glass capillary tube, waiting for the induced response to subside, and then inserting a chisel-ended wire into the capillary tube and gently rotating it along its long axis. By this procedure, the surface of the cervix was gently scraped without additional stretching or rotating of the vaginal wall. Action potentials in the pelvic nerve were readily induced by this cervical stimulation and this response was not contaminated by stimulation of the external genitalia (Fig. 1C).

The sensory field of the genitofemoral nerve (Fig. 1B) was complementary to the pudendal field. Except for overlap in the area of the clitoris, genitofemoral activity was stimulated from regions rostral to the pudendal field (Fig. 1B). In contrast to the activity in the pudendal nerve, the units in the genitofemoral (Fig. 1D) and pelvic nerves (Fig. 1C) did not adapt rapidly. In all three nerves, very little spontaneous activity was observed.

These findings provide evidence that a hormone can influence a peripheral sensory mechanism. At this point, we can only speculate as to the nature and significance of the increase in size of

the sensory field of the pudendal nerve. Estrogen may change the mechanical properties of the tissue surrounding the hair follicles, thereby changing the movement detection by the sensory nerve endings; it may increase the number or sensitivity of nerve endings, at least at the borders of the receptive fields; it may sensitize a central state which in turn exerts a centrifugal effect on the pudendal fibers by way of another nerve; it may change the blood supply to the receptors, thus altering their sensitivity. Since mechanical stimulation of the perineum potentiates the lordosis response (6), an expansion of the response region could facilitate a lordosis response to even misdirected copulatory thrusts by the male. In this way, estrogen may function as a motivational variable which increases the size of a genital sensory field in a manner analogous to the way in which hypothalamic stimulation increases the size of somatic sensory fields (13).

Our finding that the pudendal nerve receives input from the clitoral sheath and perineum is consistent with that of Cooper (5) who demonstrated input from the penis and adjacent perineum. Although Todd (14) reported input to the pudendal nerve from the perianal skin, anal lining and anal sphincter in cats, we recorded no definite response in the pudendal nerve from stimulation of either these regions or the vaginal orifice in rats. Also, Bradley and Teague reported no response in pudendal, urethral, or pelvic detrussor nerves to stimulation of pudendal anal afferents in cats (15).

The finding that the pelvic nerve receives input from the genital tract is consistent with previous reports based upon behavioral and functional (7, 16), anatomical (17), and neurophysiological (18) evidence (except that the latter study did not report input from the cervix). The results showing that the pelvic nerve also receives input from

the rectum helps to account for the effectiveness of rectal probing in facilitating lordosis and inducing immobilization in rats; both effects are also elicited by probing the vaginal cervix (6).

*Note added in proof:* Kow and Pfaff (20) have also found that the size of the sensory field of the pudendal nerve was increased by systemic estrogen treatment.

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9. The electrodes were insulated with Teflon except at the hook; they were placed 1–2 mm apart in each nerve. Action potentials were recorded through a Grass HIP5A high impedance probe, amplified through a Grass 7P3 AC amplifier, and filtered through a Kopf spike filter. Action potentials were displayed on a Tektronix 564 storage cathode-ray oscilloscope and were monitored with a Grass AM 4 audio monitor. The nerve remained moist since body fluids continuously seeped into the recording region; we periodically blotted the region adjacent to the nerve with cotton wicks. To determine the boundaries of the responsive region, a flexible plastic ruler was used to prevent inadvertent brushing of responsive regions. Alternatively, movement of responsive regions was prevented by holding the skin well outside the responsive region and brushing toward the responsive region. The maximum sensory field dimensions were determined by brushing the fur, and when the boundary was reached, the skin was scratched. This stimulus occasionally generated action potentials in the nerve at a distance of a few millimeters beyond the boundary of the long fur. The clitoral field was found by brushing the clitoral sheath and adjacent skin. There was no significant correlation between latency (days) from ovariectomy to testing and the area of the sensory field in the uninjected rats ( $r = .42$ ,  $P > .05$ , one-tailed); and there was no significant correlation between number of estrogen injections and area of sensory field in the hormone-treated rats ( $r = .43$ ,  $P > .05$ , one-tailed). Anesthetic was injected intraperitoneally on the side opposite to that used for recording. The pudendal and pelvic nerves were exposed by way of a ventral approach under a dissecting microscope, by making a 6-cm incision 1 cm lateral to the midline, and locating the internal iliac vein. The electrodes were inserted into the nerves just caudal to the region where they diverge. The genitofemoral nerve lies superficially on the abdominal muscle wall and recordings were made from this nerve at about the same anterior-posterior level as the other nerves. Each rat was taped in a supine position to an electrode carrier base so that the heel of the left leg was 3.5 cm from the midline as shown in Fig. 1A and 1B. A series of dots was drawn on the fur 1 cm apart with solvent blue 38 stain (Eastman) dissolved in absolute methanol. These extended in two parallel rows, one from the tip of the clitoral sheath to the knee joint, the other 1 cm below the clitoral dot. The genital region and grid were photographed with a Polaroid MP3 camera and sensory fields were plotted directly on the photographs. All experiments were performed blind, and on any recording day two experimental and two control rats were selected for study. Each day, we did not know the treatment of any rat until all rats had been studied. Two observers plotted the extent of each receptive field. Since the genital tract could be observed in approaching the nerve for positioning the electrodes, a technician, who was not involved in any other aspect of the experiment, performed the initial part of the dissection. The technician carefully obscured the uterus and vagina with saline-soaked gauze pads. At the end of each day after recording the results, all uteri were dissected free and weighed in order to confirm the effectiveness of the estrogen. The range of uterine weights was 361 to 615 mg in the estrogen-treated rats and 69 to 125 mg in the noninjected rats. The outline of each sensory field was drawn on the calibrated photograph of each rat, and traced twice with a Keuffel and Esser model 62 0015 compensating polar planimeter. By comparing this mean area reading with the mean reading obtained by measuring the area of a 20 by 20 mm square based on the calibration of each photograph, the actual area of the sensory field was calculated.
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11. In a few cases the field appeared to extend slightly across the midline but this may have been due to a combination of high sensitivity and slight mechanical disturbance extending beyond the truly sensitive region.
12. In the rat, the clitoral sheath is an approximately 4-mm protuberance, the base of which is continuous with the abdomen and the apex of which contains the urethral opening and points toward the tail.
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## Neurotrophic Effect on Isolated Chick Embryo Muscle in Culture

*Abstract. Acetylcholinesterase activity in cultures of dissociated skeletal muscle prepared from the thigh muscle of the 10-day-old chick embryo was increased by the presence of innervating spinal cord explants, spinal cord explants in a parabolic environment, and by media containing brain-spinal cord extract.*

Numerous experimental observations have indicated that motor neurons have important trophic effects on muscle (1). Recent reports indicate that many in vivo observations of trophic influences of neurons can be reproduced in cells grown in tissue or organ culture. Peterson and Crain (2) observed that neuritic contacts between explants of spinal cord and muscle enhanced muscle development in explants of fetal rodent skeletal muscle. Kano and Shimada (3) found that, when the dissociated thigh muscle of the embryonic chick was grown in culture together with explants of spinal cord, the formation of functional neuromuscular junctions re-

stricted the acetylcholine-sensitive area of muscle cells to the junctional region. In noninnervated fibers the whole surface was sensitive to acetylcholine. Lentz (4) found that sensory ganglia in direct contact with organ cultures of forelimb muscle from adult newts produced a delay in the decrease in cholinesterase activity that normally occurred as a result of denervation. A similar effect was produced when the sensory ganglia were separated from the muscle by a Millipore filter. The addition of homogenates of nerve tissue to the culture media also produced greater activity of muscle cholinesterase than occurred in untreated muscle cultured