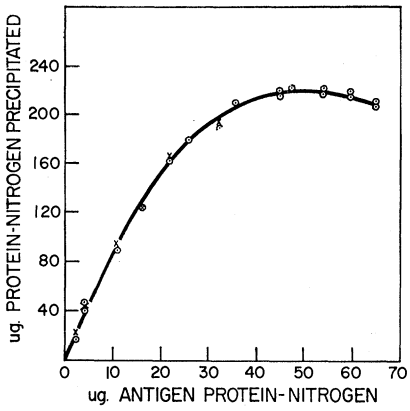

Fig. 2. Comparison of the Kjeldahl (×) and bromsulfalein (⊙) methods for measuring precipitate as a function of quantity of antigen (human gamma globulin) in the precipitin reaction with rabbit antiserum. Values from the Bromsulfalein method were multiplied by 100 to make them comparable to the Kjeldahl data.

Black 10B and Orange I exhibited approximately the same change in absorbance per unit protein over their linear ranges as bromsulfalein, their linearity was confined to a short range, making bromsulfalein preferable for analytical work.

The agreement between the Kjeldahl method for determining the amount of antigen-antibody precipitate and the bromsulfalein method is apparent in Fig. 2. The procedure for the latter method, which follows, differed from that for the former in the use of volumes 100 times smaller. Ten microliters of rabbit antiserum to human gamma globulin was placed in each of a series of glass tubes 27 mm long and of 4-mm inner diameter. Ten microliters of the gamma globulin in 0.9-percent sodium chloride (10 dilutions were used over the range 4.3 to 47 mμg/μl) was added to each tube and mixed. Each concentration of globulin was set up in triplicate, and blanks were included in which 0.9-percent sodium chloride was substituted for the globulin solution. The tubes were held at 37°C for 1 hour and transferred to a refrigerator for 7 days' storage at 2°C, during which time the precipitates were carefully resuspended once each day by gently tapping the tubes so as to prevent precipitate from lodging on the walls above the liquid. The tubes were then centrifuged at 2°C for 1 hour at 3800*g* in a microcentrifuge (*6*). The supernatant fluid was withdrawn by a micropipette and discarded, 15 μl of cold 0.9-percent sodium chloride was added, and the precipitate was resuspended by "buzzing"—that is, vibrating the vessel by touching its side near the bottom to a flattened nail spinning in the chuck of a high-speed hand drill—and then recentrifuged as before. A second washing of the precipitate was carried out in the same manner, and then the precipitate was used for protein-nitrogen measurement. A check of the procedure revealed that a third washing was without influence.

The "micro" Kjeldahl analysis of protein-nitrogen was conducted in the standard fashion. The dye procedure was carried out as follows. The precipitates in the tubes were dried in a vacuum desiccator. The protein was dissolved in 20 μl of 1*N* sodium hydroxide by mixing, letting the mixture stand for 30 minutes, and again mixing. Fifty microliters of bromsulfalein reagent (1 ml of 5-percent dye plus 100 ml of 1*N* hydrochloric acid plus 50 ml of 1*M* citric acid plus distilled water to a final volume of 250 ml) was added and mixed. The tubes were centrifuged at room temperature for 5 minutes at 3800*g*. Sixty microliters of supernatant was withdrawn and added to 1 ml of 0.1*N* sodium hydroxide. After mixing, the absorbance was measured at 580 mμ, and the protein-nitrogen was calculated as described earlier (*2*).

To test the precision of the dye method, the entire procedure was repeated nine times; a single gamma globulin solution was used (43 mμg/μl), and all volumes were reduced to 1/10 the volumes used in the earlier tests—that is, 1 μl of antiserum and 1 μl of gamma globulin were used. Tubes 27 mm long and of 2.5-mm inner diameter were employed. A mean value of 233 mμg of protein (standard deviation and error, 10.4 and 4.7 percent, respectively) was obtained.

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6. The microcentrifuge used in these experiments was provided by the Microchemical Specialties Co., Berkeley, Calif.

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Hypothalamic Lesions and Sexual Behavior in the Female Rat

Abstract. This study reports selective effects on the mating pattern of the female rat of partial destruction of the hypothalamus. Independent neural control of the ovarian cycle and of the mating response are demonstrated. Both depressed and augmented female sexual activity are reported.

Several studies have suggested the importance of the hypothalamus in the regulation and control of sexual behavior (*1–5*). Most previous work reports diminution or abolition of mating behavior following interference with hypothalamic structures. The effect is usually ascribed to disturbances in the production of the gonadotrophins.

This study (*6*) indicates that sexual behavior is differentially affected by small lesions in discrete hypothalamic regions; some lesions augment sexual activity. Moreover, the effect is not wholly hormonal; central neural mechanisms are immediately involved.

Forty-five female rats surviving all operative procedures were divided among two major groups, spayed and nonspayed. Each group consisted of four subgroups: animals with lesions in the anterior, central, and posterior hypothalamus (designated "operated" subgroups), and animals without lesions. Lesions were stereotactically placed 1 mm lateral to the midline and 1 mm dorsal to the ventral surface of the brain, and at Krieg coordinates 58 and 59 (anterior), 56 and 57 (central), and 54 and 55 (posterior). Direct current of 1.5 to 2.0 ma, lasting for 10 to 15 seconds, was used to make the lesions. Locations of lesions were subsequently verified histologically.

Estrus was induced in the spayed rats once weekly for 3 weeks by treatment with diethylstilbestrol and progesterone in sequence (*7*).

Females were tested for mating responses with male rats preselected as vigorous maters. Tests were conducted daily for 10 minutes for each female. Tests eliciting no male response were repeated the same day with another test male. All tests were conducted in transparent cages, which served as the male's home cage.

Female mating behavior was defined in terms of the characteristic stereotyped lordosis response, a concave arching of the back upon approach and mounting by the male. Lordosis frequencies were tallied and appear as "responses" in Table 1.

Daily vaginal smears were taken. Smears were dichotomized into diestrus (leukocytes only) and estrus (presence of epithelial cells). This unconventional,

Table 1. Comparison of vaginal smear and mating behavior of spayed and nonspayed, operated, and unoperated female rats. A.H.L., anterior hypothalamic lesions; C.H.L., central hypothalamic lesions; P.H.L., posterior hypothalamic lesions.

Item	(1) No.	(2) Total days tested	(3) Mean days diestrus	(4) Mean days mating, diestrus	(5) No. responding	(6) Mean responses in diestrus	(7) Mean days, estrus	(8) Mean days mating, estrus	(9) No. responding	(10) Mean responses in estrus
					Spayed					
Control	8	195			1				8	
Mean			4.4	0.25		1.0	20.0	9.7		7.8
Range		20–29	2–9	0–2		0–8	16–23	3–13		1.6–15.3
A.H.L.	4	48			1				4	
Mean			2.8	0.25		2.0	9.3	1.5		27.3
Range		8–14	2–4	0–1		0–8	6–12	1–3		21–41
C.H.L.	3	40			1				0	
Mean			3.2	0.33		5.6	9.3	0		0.0
Range		12–14	3–6	0–1		0–17	7–11	0		0
P.H.L.	2	28			2				2	
Mean			4.5	1.5		21.5	9.5	1.5		19.0
Range		14–14	6–9	1–2		11–32	8–11	1–2		16–22
					Normal					
Control	10	139			1				9	
Mean			6.4	0.1		0.7	7.9	1.6		12.3
Range		9–18	1–11	0–1		0–7	5–12	0–3		0–24.5
A.H.L.	5	105			5				5	
Mean			13.4	1.2		12.8	7.6	1.2		12.5
Range		21–21	11–16	1–2		1–24	5–10	1–2		3–21
C.H.L.	6	125			1				0	
Mean			10.8	0.17		4.1	10.0	0		0.0
Range		20–21	0–18	0–1		0–25	3–21	0		0
P.H.L.	4	96			3				4	
Mean			14.8	3.3		14.5	9.3	4		14.7
Range		21–25	10–19	0–5		0–23	6–15	1–9		4–21.5

but conservative, classification was adopted to reduce ambiguity in interpretation of vaginal stages.

There was a tendency for spayed, hormone-treated animals without lesions to spend less time in diestrus (column 3). They resembled the normal control animals, however, in failing to mate appreciably during diestrus (column 5). Because the spayed animals spent more time in estrus, they also mated more frequently during estrus, but average responses per test (column 10) were less for the spayed than for the normal animals. All spayed subgroups had a greater percentage of days in estrus than their nonspayed counterparts, because of the exogenous hormones.

Although central hypothalamic lesions in the region of the posterior border of the optic chiasm diminished or abolished the female mating response in both groups, there was no marked tendency to produce constant vaginal cornification ("estrus"), as was found in other studies (*2, 4*). Of the nine animals with central hypothalamic lesions, only two ever mated. Both of these responses occurred while the animals were clearly in a diestrus vaginal condition. The mean response frequency for each of these cases was higher than it was for the unoperated animals in diestrus but lower than it was for the unoperated animals in estrus. None of these nine animals ever mated in estrus.

Posterior hypothalamic damage in the pre-mammillary region yields a very different result. Five of the six animals with this lesion mated in diestrus. The average response frequency of animals in this condition exceeded that of the unoperated animals in estrus, and equalled or excelled the rate for the same animals during estrus. All six animals mated at least once in estrus, and at a mean rate greater than the control rate.

Anterior hypothalamic lesions in the preoptic area gave effects on mating behavior similar to those of the P.H.L. group. The diestrus mating responses of the spayed groups are the only exception. The figures for the latter animals resemble more the performance figures for unoperated animals. Estrus mating for spayed and nonspayed animals with anterior hypothalamic lesions was normal or better. Diestrus responses for nonspayed animals are augmented.

The results indicate a more specific localization of function within the hypothalamus than was previously suspected. The decrement in female mating in the group with central hypothalamic lesions suggests interference with a neural mechanism necessary to sexual receptivity. That this effect is independent of hormonal factors is clear from the data of the groups receiving exogenous hormones. The absence of persistent vaginal cornification in our study substantiates this view. Our lesions were considerably smaller than those used in most earlier studies.

One possible explanation of the relative independence of neural and hormonal factors is that regions controlling the endocrine cycle are too widely distributed to be entirely destroyed by small lesions, and thus surviving tissue can maintain the function. Disruption of any considerable part of the mechanism involved in the complex act of mating, however, may well abolish the capacity for the performance. Thus control of the gonadotrophins may be topographically more diffuse in the hypothalamus than the related control of sexual activity; or the latter may require greater integrity of the system.

That there is a system in the female rat which opposes or inhibits receptivity is apparent from the data for animals with posterior hypothalamic lesions and probably from the data for animals with anterior hypothalamic lesions as well. These influences may well be related to others originating in more remote areas, as reported for the amygdala (*8*) and the cortex (*9*).

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