Effects of Posterior Hypothalamic Lesions on Sexual Maturation of Immature Female Albino Rats. (25461)

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Neuroendocrine investigations demonstrated that hypothalamic lesions will impair mechanisms for normal maturation of the prepuberal rat. Bogdanove(1) and Donovan (2) have shown that precocious sexual development can occur in immature female rats with anterior hypothalamic lesions. Such effects may be due to either release from a gonadotropic inhibiting system, or activation of a gonadotropic stimulatory mechanism. Although the underlying processes involved in initiation of puberty are relatively obscure, the present belief is that lack of hypothalamic stimulation delays puberal onset, since, in the sexually infantile rat, the pituitary is not too immature for, nor its target organs refractory to, gonadotropic hormone secretion. Transplantation of immature rat pituitaries to base of brain of their hypophysectomized mother restored cyclic estrus function before puberty in the donors would have been effected(3), indicating that prepuberal hypophysis awaits hypothalamic stimulation. Investigation of the possibility that posterior hypothalamic damage may cause a delay in onset of puberty is the object of this study.

Methods and materials. Forty-five, 21-day old albino female rats (Holtzman) were divided into 4 groups. Group A (12 animals) was a normal control group, Group B (12 animals) was the sham operated control group, Group C (11 animals) consisted of lesioned subjects, and Group D (10 animals) was a normal control group sacrificed at 21 days of age. Animals were allowed tap water and Purina laboratory chow. *ad lib.* Body weights were recorded every 5 days. All operations were performed between days 21 and 25. Under Nembutal anaesthesia (2 mg/50 g rat, I.P.), a 23 ga wire electrode was inserted, according to Krieg(4) coordinates 53-56, 83-84, to a depth of 0.5-1 mm from base of skull. Direct current of 5 ma for 15 sec was employed to bilaterally destroy the area from the mammillary body to the ventromedial nucleus. As soon as vaginae opened, smears were taken for 3 to 4 weeks to identify cycling activity. At the first such smear, one rat from each of Groups A and B, and 3 from Group C was placed in a cage with a virile, 100 day old male, for mating. All rats were observed for behavioral changes. Groups A, B, and C were sacrificed at 65 days of age. Fresh weights of pituitaries and reproductive organs (total weight of ovaries + oviducts + uteri + vagina) were recorded. Statistical analysis of data was by Student's "t" test. A probability of ≤ 0.05 was accepted as statistically significant.

Results. Histological examination of lesioned brains revealed extensive destruction of the area from mammillary body to VMN. Results are summarized in Tables I and II. Animals with hypothalamic lesions showed a definite inhibition of reproductive organ growth. Average reproductive organ weight of 5 lesioned animals whose vaginae never opened was 93.9 mg, less than 1/5 the average recorded for normal controls. Although pituitaries of lesioned animals were considerably smaller than those of normal controls, they were comparable in weight to those of the sham operated group, making it difficult to interpret reproductive organ size in terms of pituitary weight alone.

Vaginae of Groups A and B opened between days 34 and 35, with a cycling pattern established, on the average, 6 days after opening. In contrast, vaginal opening of Group C was delayed until the 52nd day. This delay is especially significant since the group includes 5 animals (Group C_2) whose vaginae were still closed at autopsy. These animals had the smallest reproductive organ and pituitary weights of the entire lesioned group. All Group C animals exhibited abnormal deposits

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TABLE I. Means and Standard Errors of Body Weights, Reproductive Organs, Hypophysis, Vaginal Opening, and Cycling in Normal, Sham Operated, and Lesioned Female Albino Rats.

	No. of rats	Body wt (g)		Reproductive organ wt (mg)		Hypophysis wt (mg)		Day vagina opened	Cycling began, days post vaginal opening
		21 days	65 days	21 days	65 days	21 days	65 days		
Group A, normal control	12	48.0 ± .8	191.5 ± 3.2		552.5 ±65.1		8.12 ±1.14	35.0 ± .6	6.0 ±.4
Group B, sham control	12	42.0 ±1.2	188.9 ±10.4		604.6 ±75.8		3.70 ±1.00	34.0 ±1.4	6.0 ±.4
Group C, lesioned	11	42.0 ±1.7	180.9 ± 7.4		213.4 ±60.9		2.01 ± .33	52.0 ±3.5	Anestrous
Group C_1, lesioned vaginae opened	6	42.0 ±3.0	183.5 ± 4.0		332.0 ±29.0		2.28 ± .77	40.0 ±2.1	"
Group C_2, lesioned vaginae never opened	5	42.0 ±4.2	178.8 ±23.6		93.9 ±13.8		1.75 ± .50	Unopened (at 65 days)	
Group D, normal controls 21 days old	10	42.0 ±1.4		66.64 ±1.35		.823 ±.177			

of adipose tissue in which kidneys, adrenals and unvascularized reproductive organs were deeply embedded. That such accumulation of fat exceeded that of the 2 control groups, and that large lower abdominal bulges were evident, suggested imminent obesity. One lesioned animal exhibited definite obesity, weighing 231 g. Females from Groups A and B became pregnant, bearing an average of 12 fetuses. The 3 animals from Group C when tested never became pregnant.

Of 11 animals in Group C, 5 had vaginae that never opened, 5 were diestrus and gave continuous leucocyte smears, 1 was in constant estrus, and gave continuous cornified smears (this animal failed to mate).

Marked behavioral alterations were observed in the lesioned animals. Post-operatively, the rats became extremely vicious and belligerent. Two of the weaker surviving animals were eaten alive. Continuous hostility was exhibited up to time of sacrifice. Pain and post-operative discomfort were ruled out as possible stimuli since the sham operated group displayed no such behavior, and normal rats of such a young age are quite docile and inquisitive. Food intake of lesioned animals was reduced during the first 2 post-operative weeks. Definite hyperphagic behavior was indicated by day 50, at which time the bulging, pear shaped lower abdomen became evident.

FIG. 1. Comparison of reproductive organs of normal control (left), sham-operated (center) and lesioned rat (right) at 65 days.

TABLE II. Table of "t" Values for Normal, Sham Operated and Lesioned Female Albino Rats.

Groups compared	Reproductive organ wt	Body wt (65 days)	Hypophysis wt	Day of vaginal opening
A & B	.52†	.024†	2.9 *	.6 †
A & C	3.77*	.16 †	5.00*	4.5 *
B & C	4.00*	.05 †	1.50†	4.5 *
A & C_1	3.10*	1.57 †	4.30*	2.62‡
A & C_2	6.60*	.022†	4.16*	
B & C_2			1.71†	

* P ≤.01 † P ≥.05 ‡ P ≤.02

Discussion. Fig. 1 shows the striking difference in reproductive organs of a normal control, sham operated and effectively lesioned animal. The reproductive organ immaturity and prolonged vaginal closure infers that the initiating mechanism for estrogen secretion was absent. Reproductive organ growth and maintenance of functional estrous cycling, as well as vaginal opening "on time," are depen-

dent upon this particular ovarian hormone. Furthermore, the fact that lesioned animals whose vaginae opened after considerable delay exhibited a constant anestrous state, suggests that the hypophysis was incapable of elaborating or secreting those hormones responsible for initiation and maintenance of sexual cycling.

On the other hand, rats with anterior hypothalamic lesions have exhibited precocious puberty(1,2) and persistent estrus(5,6,7). Correlation of all observations suggests the possible existence of a dual "gonado-hypophytropin" controlling system: 1) an inhibitory mechanism located in the anterior hypothalamus, and 2) a stimulatory one located in the posterior hypothalamus. Activity of these 2 hypothetical "sex centers" should determine net gonadotropic activity of the hypophysis, and influence time of puberal initiation. The effects of circulating gonadotropic and ovarian hormones upon hypothalamic function must also be considered, although further discussion of such feedback mechanisms is beyond the scope of this study. In view of previous evidence, the hypophysis is, theoretically prepared to produce its gonadotropins at a prepuberal age. The reason for puberty not normally occurring earlier may be due to a differential immaturity of the hypothalamus itself.

Physiological evidence for ventromedial nuclei destruction was indicated by the obese patterns in these animals, confirming reports of Brobeck(8). It is interesting to note the similarity between delayed puberty, sexual infantilism and obesity resulting from posterior hypothalamic destruction and the characteristic signs of Frohlich's syndrome. In effect, Frohlich's syndrome has been experimentally produced in these animals.

Summary. Eleven 21-day old female albino rats (Holtzman) were subjected to bilateral lesions of the area from the mammillary body to the ventromedial nucleus. These animals showed definite signs of delayed puberty, reproductive immaturity, viciousness and imminent obesity. Five had vaginae that never opened. It appears evident that in the prepuberal rat, factors (neurosecretory substances), which prod the pituitary into a functional secretory state, and which permit the mammal to attain reproducive maturity, are not present, or, are in a state of preparation. The results indicate the importance of hypothalamic function to puberal onset, and to the structural and physiological success of the reproductive-sex complex.

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Effect of Methionine upon Ethionine Intoxication of the Rat.* (25462)

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Ethionine is an analogue and metabolic antagonist to methionine(1,2,3), and ample evidence has been presented that the features of ethionine intoxication including cancer are prevented by administration of methionine(4). Nevertheless, the possibility of a toxic effect

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