FOREBRAIN REGULATION OF OVARIAN CYCLE IN RATS : EVIDENCE FOR A DUAL CONTROL*

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Summary: Tiny, bilateral, symmetrical lesions were made stereotaxically in septum, area parolfactoria, thalamus, head of caudate nucleus and amygdala of adult female albino rats with regular 4 day estrus cycle. The estrus cycles were studied for 2 months post operatively. At the end of the study, the brains were taken out for confirmation of sites of lesion and ovaries and uteri for histological changes. Majority of the rats with lesions of medial septum, antero - and dorsomedial thalamus, anteromedial amygdala and head of caudate nuclei showed preponderance of estrus. Lesions of nucleus accumbens, area olfactorius and lateral amygdala, favoured production of met and diestrus. Histological appearance of ovaries and uteri presented a variable picture depending on the site of lesion rather than the associated vaginal cytology. These observations suggest that both facilitatory and inhibitory neural substrates, influencing ovarian cycle, are present in the forebrain, and they produce differential effects on vaginal estrus and ovarian and uterine histology.

Key words: forebrain ovarian cycle limbic system
forebrain lesions and ovarian cycle

The role of hypothalamus in modulating the activities of anterior pituitary which in turn influence the activities of the gonads, is well recognized (7, 9, 10). Lesions of anterior hypothalamus and suprachiasmatic regions have been shown to produce constant vaginal estrus (1, 6, 22, 23) where as lesions in basal tuberal region of the median eminence area lead to constant diestrus (23). Details of hypothalamic localisation and analysis of these controlling mechanisms have been recently summarised by Flerko (5). Evidence is also accumulating to implicate other regions of the brain in influencing the socio-sexual behaviour of the animals (19). In a series of studies in the male squirrel monkeys, MacLean and his associates (3, 12, 13, 14) have reported that systematic stimulation of midline cortical and brainstem structures from the frontal pole to the level of medulla lead to penile erection, ejaculation, scratching and other manifestations of sexual behaviour. Ejaculation in rats has been reported on stimulation of medial forebrain bundle (11, 16).

Everett *et al.* (4) attempted to stimulate the various forebrain structures with irritative focal lesions formed by electrolytic deposition of iron from stainless steel electrodes. With such a mode of stimulation, ovulation was elicited from anterior hypothalamic area, medial

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preoptic region and septal complex, but no changes were obtained from caudate-putamen, anterior olfactory area, olfactory tubercle, lateral preoptic area and dorsal thalamus. These observations were, however, limited to the effects seen immediately after producing irritative lesions. The present study was, therefore, designed to see the long range effects of lesions in forebrain structures on estrus cycle and also the concommittent changes in ovarian and uterine histology.

MATERIALS AND METHODS

Eighty six female adult albino rats with regular 4 day estrus cycles, studied by vaginal smear technique and observed for about a month, were taken for this stndy. Following the co-ordinates of de Groot atlas (2), tiny, discrete, bilaterally symmetrical electrolytic lesions were attempted stereotaxically in various forebrain structures like septum, area parolfactoria, thalamus, amygdala, and the head of the caudate nucleus. Lesions were produced by passing 2 mA anodal d.c. for 20 sec through a stainless steel wire which was insulated except for 1 mm at the tip. The estrus cycles were studied for two months post operatively. At the end of the study the animals were anaesthetised and sacrificed by intracardiac perfusion of 0.9% saline followed by 10% formaline. A thorough postmortem examination was done for any gross pathological changes. As a routine in all the animals, the brain was taken out for confirmation of sites of lesions and the ovaries and the uterus for histological examination.

Urine samples of some of the lesioned rats in continuous estrus phase and a few with irregular cycles, were collected for 24-48 hours. These urine samples were later acidified, concentrated at high temperatures and extracted with ether and/or Butanol in a liquid - liquid extractor. The extracts were injected into some of the nonlesioned controls, as well as into overiectomized rats and their vaginal smears studied for any cornification.

The changes observed in morphological pattern of reproductive tract and the sexual behaviour of the rat, are generally known to move in similar direction during proestrus (P) and estrus (E), but are different from the direction of changes observed during metestrus (M) and diestrus (D). The values of proestrus and estrus, therefore, have been pooled together and compared with the pooled values for metestrus and diestrus. Normally the ratio of the two i.e., $(P+E)/(M+D)$ is 1 in the rat with a 4 day regular estrus cycle. This ratio has been named as Estrus Index (E.I.). Such an expression of E.I. has been found quite useful to classify the irregular cycles observed after lesions of various brain structures. The E.I. of more than one suggests preponderance of estrus phase whereas E.I. below one suggests preponderance of diestrus.

RESULTS

Serial histological brain sections revealed that in majority of the animals lesions were discrete, symmmetrical and well localised. Such lesions were found in medial (A 8.6) and lateral (A 7.8) septal areas, antero-and dorsomedial thalamic nuclei (A 5.0-5.8), area olfac-

torius and nucleus accumbens (A 8.2), lateral and basolateral amygdaloid nuclei (A 5.4), head of caudate nucleus (A 9.4), and lateral hypothalamus (A 6.2) and are shown in Fig. 1. In few animals the lesions were either too small or too extensive and asymmetrical and their results are not being included in this report. Irregular cycles for 2-3 weeks immediately after the lesions was a routine feature observed in most of the rats. However, rats with lesions in septal, thalamic, anteromedial amygdaloid nuclei and area olfactoria, in general, showed preponderance of estrus while rats with lesions of nucleus accumbens, lateral amygdaloid nuclei, head of caudate nucleus and lateral hypothalamus showed preponderance of met and diestrus. After this initial period of 2-3 weeks, the cycles either came back to normal regular ones or were followed by continuous estrus phase or irregular phases with estrus or diestrus preponderance.

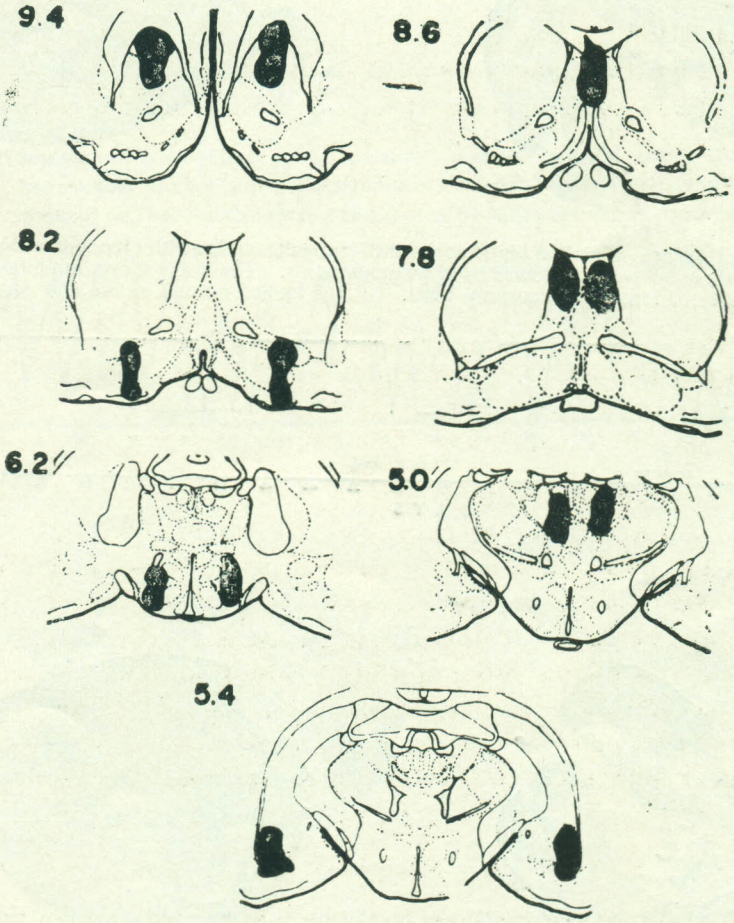
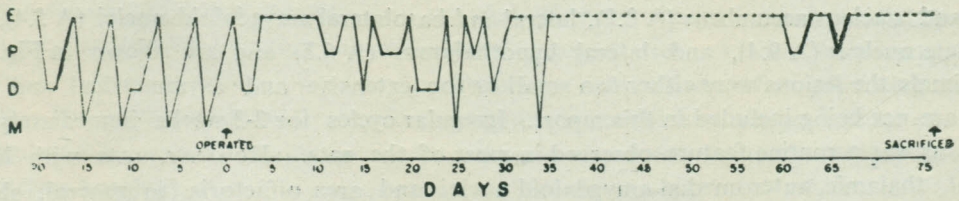


Fig. 1: Shows the sites of lesion in various groups depicted on schematic frontal sections according to de Groot's atlas. A-P coordinates are given to the left of each figure.



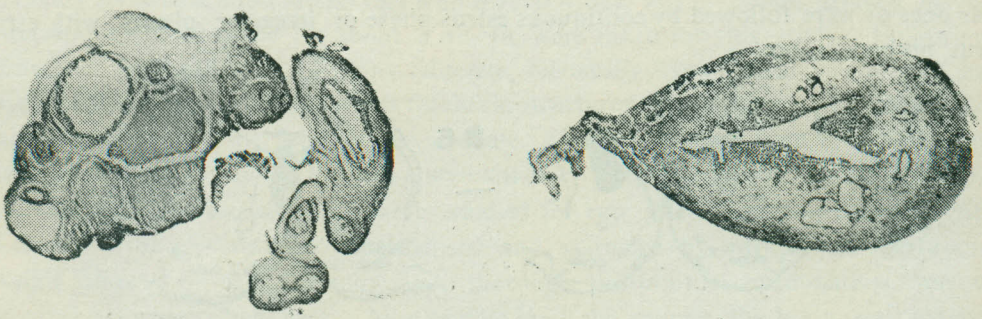
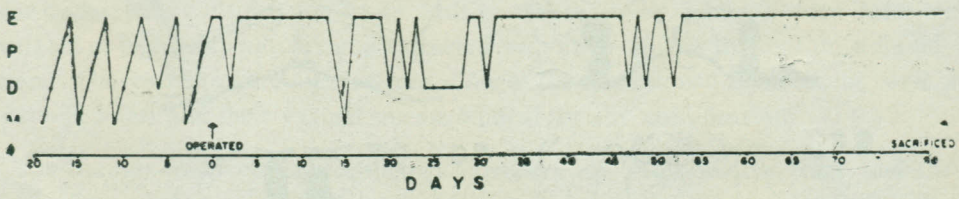


Fig. 2: Bilateral lesions of medial septal area led to irregular cycles with preponderance of estrus for the first 3-4 weeks followed by continuous estrus. The ovary shows inhibition of new follicle formation and few corpora lutea. Uterus looked normal in size and structure.



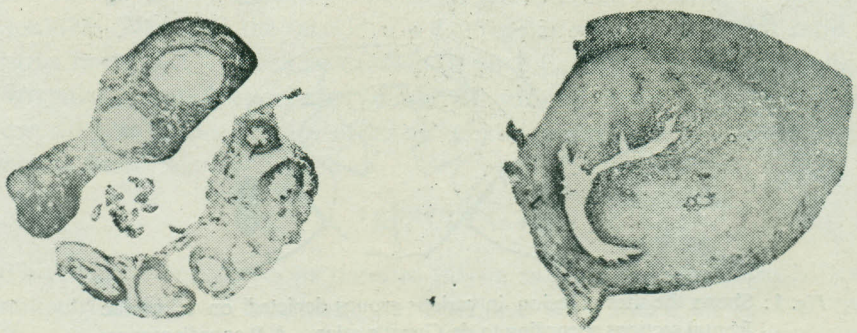


Fig. 3: Preponderance of estrus followed by continuous estrus, small sized ovary with fewer follicles and corpora lutea, and normal uterus, seen after bilateral lesion of anteromedial thalamic nuclei.

Lesions of medial septal and antero - and dorsomedial thalamic nuclei usually led to continuous estrus phase (Fig. 2 & 3). Such pholonged estrus was replaced by irregular cycles with preponderance for estrus when the lesions were a little lateral in lateral septal areas. The urine of some of the rats with medial septal lesions and showing continuous estrus, was extracted with ether and/or Butanol and injected into normal control rats as well as into ovariectomized rats. Ether extract did not produce any significant change in the estrus cycle but Butanol extract led to two peaks of cornifications in the overiectomized rat about 48 hours after the injection (Fig. 4). Similar injections into the control, unoperated

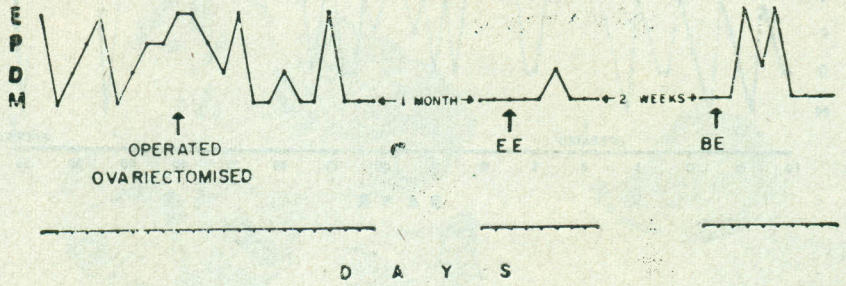
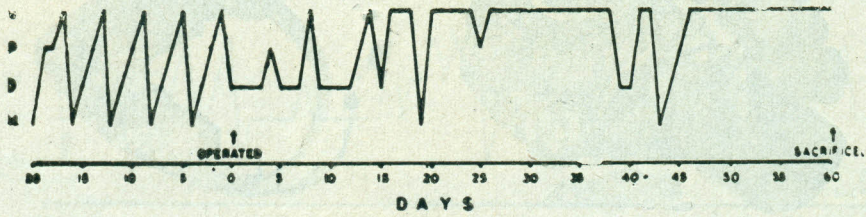


Fig. 4: Injection of ether extract (E.E.) of urine of septal lesioned rat in continuous estrus phase did not show any significant change in vaginal cytology of ovariectomized rat, but Butanol extract (B.E.) of urine led to two peaks of cornification.



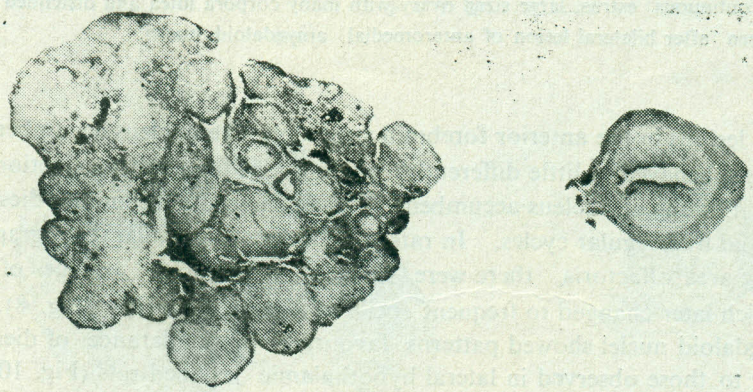


Fig. 5: Bilateral lesion of head of caudate nucleus produced preponderance of diestrus soon after operation followed by continuous estrus. The large sized ovary shows many corpora lutea, while the uterus appears normal.

rats preponed or precipitated the cornification in vaginal smears. Urine extracts of rats with lesions of thalamic nuclei and showing continuous estrus, did not produce any change in vaginal smears of ovariectomized or unoperated control rats. In some of the rats with lesions of the head of caudate nuclei, preponderonce of diestrus was seen soon after the lesion, but it changed into continuous estrus phase after 2-3 weeks (Fig. 5). Continuous estrus response was also observed after lesions of anteromedial amygdaloid nuclei (Fig. 6).

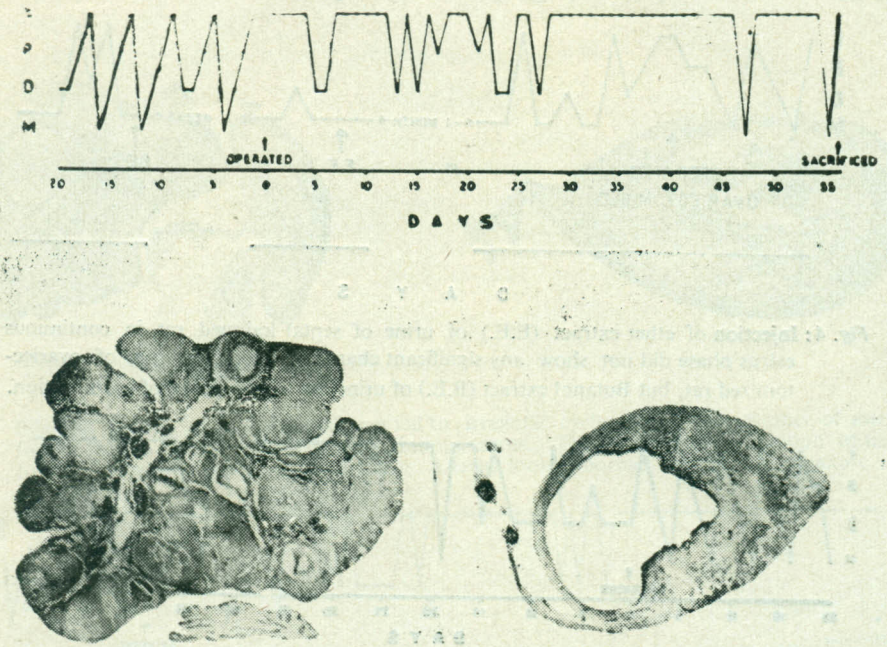


Fig. 6: Continuous estrus, large sized ovary with many corpora lutea and distended uterus, seen after bilateral lesion of anteromedial amygdaloid nuclei.

Rats with lesions in the anterior forebrain structures like nucleus accumbens and lateral amygdaloid nuclei, behaved a little differently: such lesions favoured production of met and diestrus phases. Lesions of nucleus accumbens (Fig. 7) showed continuous diestrus for first 2-3 weeks followed by irregular cycles. In rats with lesions in similar topographical regions but also involving area olfactoria, there were frequent continuous stretches of estrus in the initial phases which later changed to frequent spells of di and metestrus (Fig. 8). Lesions of basolateral amygdaloid nuclei showed patterns favouring preponderance of diestrus (Fig. 9), and were similar to those observed in lateral hypothalamic lesioned rats (Fig. 10).

The estrus Index calculated for 2 months post operatively varied from 0.4 to 7.4 and depended on the site of lesions (Table I).

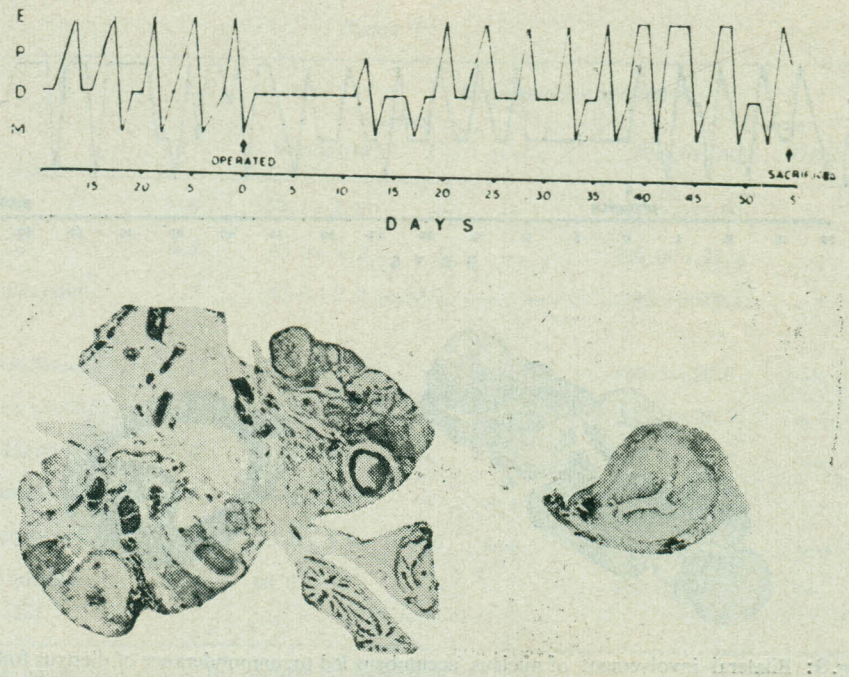


Fig. 7: Bilateral lesions of basolateral amygdaloid nuclei showed preponderance of diestrus, larger ovaries with varying number of corpora lutea and follicles and small uterus.

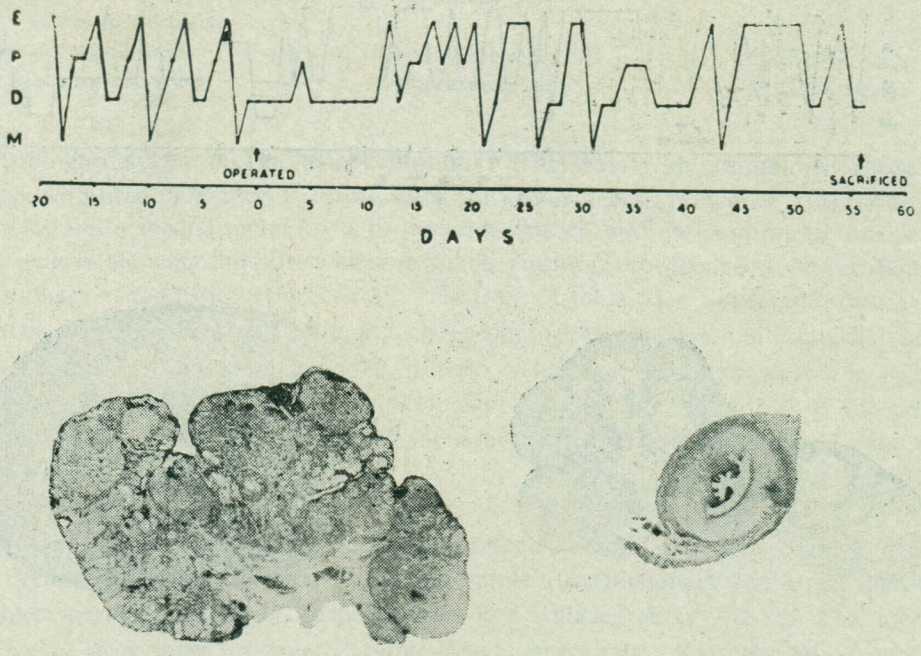


Fig. 8: Prepondenrance of diestrus, presence of both corpora lutea and follicles in the ovary and small uterus seen after lesion of lateral hypothalamus.

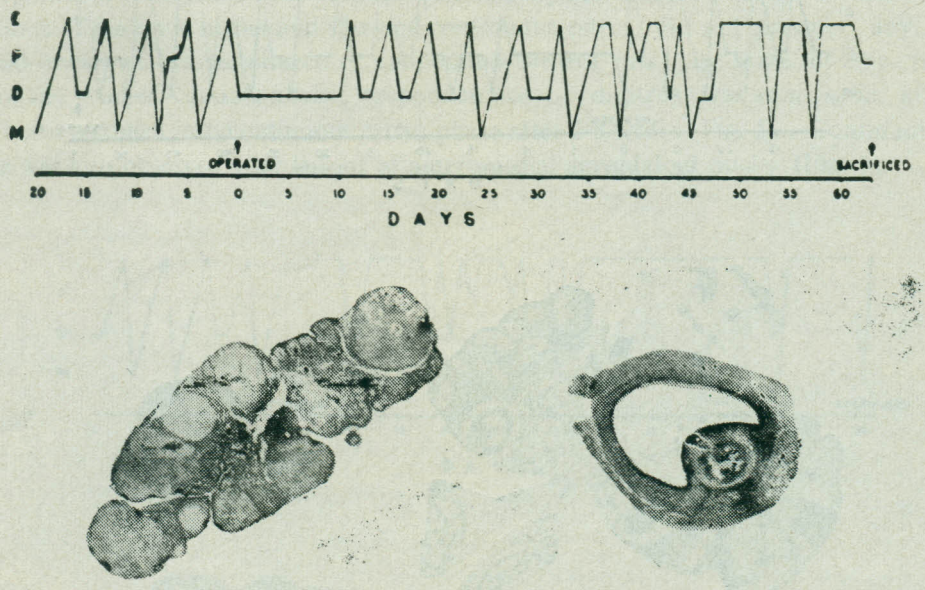


Fig. 9: Bilateral involvement of nucleus accumbens led to preponderance of diestrus followed by irregular cycles increase in corpora lutea and distended uterus.

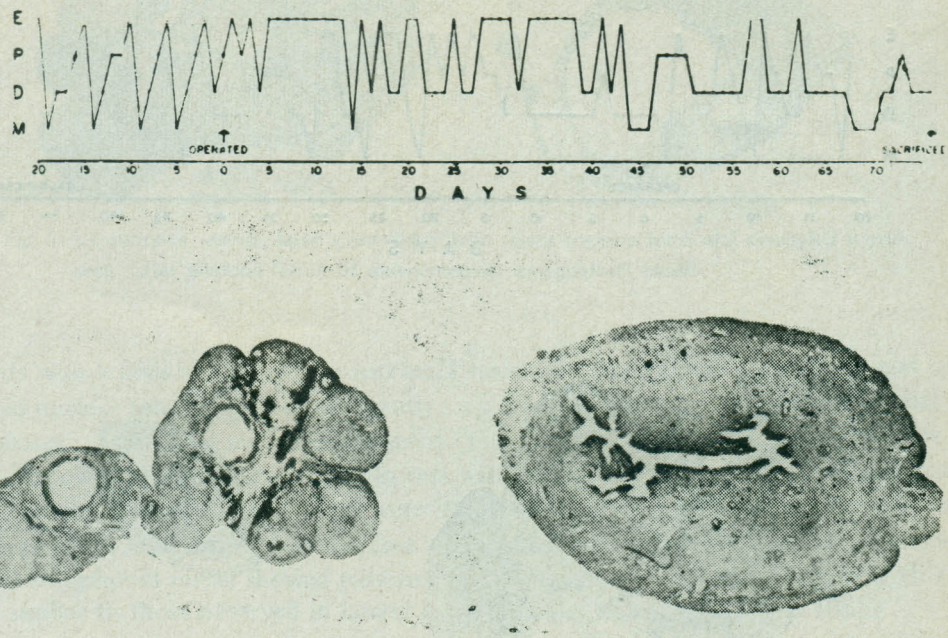


Fig. 10: Preponderance of estrus followed by preponderance of diestrus, normal ovary and large uterus' seen after involvement of nucleus accumbens and area olfactoria.

TABLE I

*Site of lesion**	*E.I.*	*Histological Appearance*					
		Ovaries			*Uterus*		
		Weight(mg)	*F*	*C.L.*	*Weight (mg)*	*Cavity*	*Glands*
Medial septum (10)	7.4	63.1±2.8	—	—	383.6±27.09	±	±
Lateral septum (7)	4.8	65.0±4.2	—	—	380.00±28.8	±	±
Antero—and dorsomedial thalamus (10)	5.2	52.0±2.8	(cystic)	— —	390.1±28.1	±	±
Head of caudate nucleus(7)	2.8	128.0±3.5	—	+++	380.5±28.8	±	±
Anteromedial amygdala(5)	5.2	131.0±2.9	—	+++	399.0±26.7	++	±
Basolateral amygdala(5)	0.4	78±0.8	±	++	367.0±30.5	—	±
Lateral hypothalamus (6)	0.8	80.0±3.8	±	++	367.6±30.5	—	—
Nucleus accumbens (8)	0.9	73.0±3.1	—	++	370.4±27.5	++	—
Area olfactoria & nucleus accumbens (12)	0.9	68.0±4.2	— (cystic)	+	410.0±28.7	±	+

* Figures in parenthesis denote the number of animals in the group

E.I. = Estrus Index

F. = Follicles; C.L. = Corpora lutea

± = Doubtful change

\+ = Slight increase ++ = Moderate increase; +++ = Marked increase

— = Slight decrease — — = Moderate decrease; — — — = Marked decrease

Histological appearance of ovaries and uteri from rats with continuous estrus or irregular cycles with preponderance of estrus, were not uniform and presented a variable picture. Rats with lesions of medial septal areas or thalamic nuclei and in continuous estrus phase, had small ovaries showing inhibition of new follicle ripening process and a few corpora lutea in various stages of regression (Fig. 2 & 3). The uteri of these rats appeared normal in size and structure. In rats showing continuous estrus phase after lesions of anteromedial amygdaloid nuclei or head of caudate nucleus, the ovaries were significantly increased in size and were full of corpora lutea with very few follicles (Fig. 5 & 6). Majority of the rats with amygdaloid lesions had distended uteri but the uteri of the caudate lesioned rats showed normal appearance.

Rats with preponderance of diestrus also presented variable histology. The ovaries of rats with lesions of basolateral amygdala and lateral hypothalamus were larger than normal and contained varying numbers of corpora lutea and follicles (Figs. 7 & 8). The uteri were usually smaller than those observed in nonlesioned control rats. Rats with lesions of anterior olfactory areas showed uteri larger than those seen in normal controls (Figs. 9 & 10). Table I,

gives the details of the site of lesions, Estrus Index, weight and histological appearance of ovarie and uteri in various groups. Fig. 11 summarises the sites of lesions producing different types o responses.

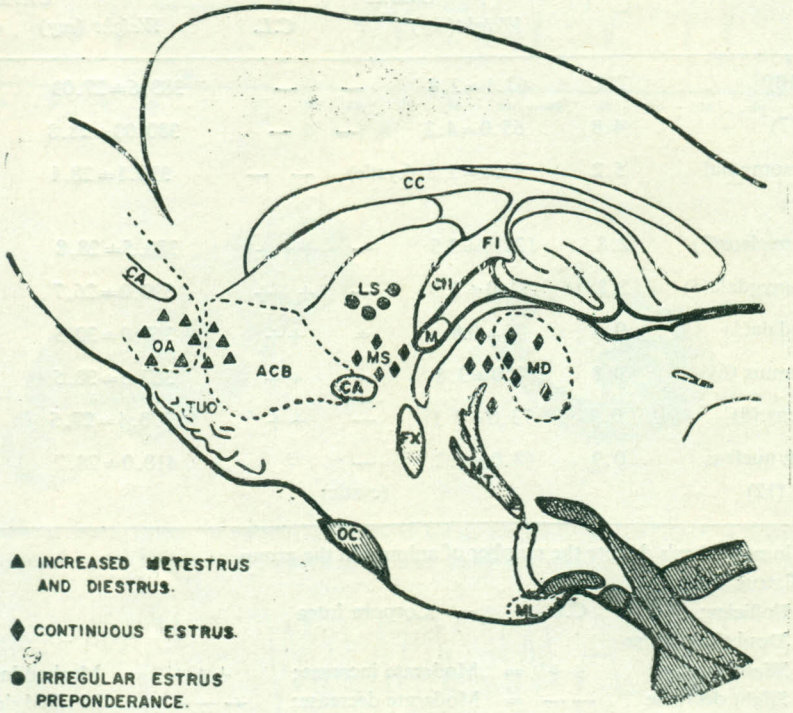


Fig. 11: Diagrammatic sketch of sagittal section of brain showing the various sites, lesions of which produced various disorders in estrous cycle.

DISCUSSION

A review of the experimental data available so far, as regards vaginal smears, morpho logical response of the hypophysis to estrogen, gonadotropic activity, state of gonads an accessory reproductive organs both in intact and lesioned female rats, does not offer one clearcut picture. However, it is generally agreed that the cyclic changes observed in vagina cytology, and in uterine and ovarian morphology are under the influence of three gonadotropin released through hypothalamic mechanisms. McCann (15) has summarized the functions o these three gonadotropins in the female rat by saying that the primary effect of follicle stimulat ing hormone (FSH) is to produce growth of the follicles because apparently it does not b itself stimulate any hormone production by the ovary. For that there is a second gonadotro pin, luteinizing hormone (LH), which in the presence of some FSH, effects further follicula growth, ovulation, estrogen secretion, and formation of corpora lutea. Then there is a thir gonadotropin fairly well established, in the rat at least and known as luteotropin or prolacti

(LTH), which is also required for the maintenance of corpora lutea and for the secretion of progesterone by the corpora lutea. Further it is also well established that the regulation of secretion of these pituitary gonadotropins is dependent on the functional integrity of hypothalamus and the hypophysis. The present study points out that besides hypothalamus, there are other central nervous structures influencing the release of these gonadotropins. These brain structures must finally be exerting their influence through the hypothalamus which is regarded as the last neuronal link in the major endocrine-motor organisation and may be compared to the "final common path" of somatic motor organization (17). Though this hypophysiotrophic area can maintain and regulate the basal secretion of trophic hormones, the cyclic variations require afferent input. The pathways needed for such cyclic variations enter from more anterior brain regions. The role of hypophysiotrophic area and its regulation has been reviewed recently by Bela Flerko (8).

Forebrain influence is not a simple facilitatory or inhibitory one but may differentially influence the secretion of the three gonadotropins. Though continuous vaginal estrus was observed after lesions of medial septum, thalamus, anteromedial amygdala and the head of caudate nucleus, the changes in ovarian histology and the effects of urinary extracts of rats of these groups did not move in the same direction. Septal lesioned rats had continuous estrus, increased level of excreted estrogen in the urine as seen from the effects of the urine extract injections into the ovariectomized rats, and inhibition of new follicular growth with few corpora lutea in the ovaries. It is likely that septal lesions suppress FSH and LH, but enhance the estrogen secretion. Non-follicular sources of estrogen have been known to exist (18) and might well account for the increased estrogen titre observed in septal lesioned rats.

Thalamic lesions produce continuous estrus, but the urine extract response is negative and the ovary has follicular cysts with significantly lesser number of corpora lutea than in septal ovary. May be, such thalamic lesions, suppress the secretions of LH and LTH more than the FSH secretion. This might also explain why Everett *et al.* (4) could not elicit ovulation by placing lesions in the thalamus though they were able to get the positive results after lesions of septal area.

The large ovaries with a number of corpora lutea and continuous estrus but practically showing no follicles after lesions of anteromedial amygdala and head of caudate nucleus, also point to the imbalance produced in the secretions of FSH, LH and LTH. The FSH secretion appears to be suppressed while LH and LTH secretions are increased after such lesions. The higher estrogen titre may be due to activation of extra follicular sources of estrogen.

Involvement of lateral hypothalamus or basolateral amygdala produce continuous diestrus for 2-3 weeks followed by irregular cycles with preponderance of diestrus. The ovaries had both follicles and corpora lutea. The uteri were usually smaller than normal. Most investigators who have produced persistent vaginal cornification in rodents with hypothalamic

lesions, have reported inhibition of corpus luteum formation in the ovary. Uterine hypertrophy can occur without change in ovarian weight and small ovaries may exist with or without enlarged uteri (1). Taleisnik and McCann (23) suggest that there is a partial defect in the LH mechanism in the animal in constant estrus after hypothalamic lesions but not nearly as severe as in the one in constant diestrus.

Lesions of area olfactoria and nucleus accumbens, led to preponderance of diestrus, normal ovaries and distended uteri. Signoret and Mauleon (21) have shown disturbances of the estrus cycle following ablation of olfactory lobes in the sow. After complete removal there was involution of the genital tract; after partial removal the degree of disturbance of the estrus was related to the amount removed. Histological appearance of the hypophysis suggested blockage of the discharge of the gonadotropins because secretory material was accumulated in the cells,—another point in favour of forebrain influence over secretion of pituitary gonadotropins.

Van Der Werff Ten Bosch *et al.* (24) reported highest pituitary and serum LH levels during late diestrus of normal cycle of the rat. They further reported that pituitary LH of rats with constant estrus as a consequence of anterior hypothalamic lesions was found to be comparable to that of the late diestrus of the normal cycle, but serum LH was barely detectable. This might lead one to think that though the production of pituitary LH after anterior hypothalamic lesions is increased, it is not released into the blood in adequate amounts. Similar conclusions concerning FSH production and release can be drawn in constant estrus rats after hypothalamic lesions (1). The constant estrus and large number of corpora lutea in the ovaries of the present series of animals after lesions of anteromedial amygdala and head of caudate nuclei suggest an increase in both pitutitary and serum LH levels. The latter may be due either to "feed back" effect of constant estrogen action continuously stimulating release of LH from the pituitary gland (5) or perhaps secondary to release of some inhibitory nervous mechanisms regulating LH secretion. Forebrain inhibitory mechanisms related to puberty, sexual behaviour and sex hormones are known to exist (20). It appears that the mechanisms for constant estrus and increased LH secretion observed in forebrain lesioned animals are different from the constant estrus and decreased serum LH produced by anterior hypothalamic lesions.

The diverse morphology of ovarian and uterine structures with continuous estrus or preponderance of diestrus after lesions of thalamus, septum, amygdala, head of caudate nucleus, area olfactoria, nucleus accumbens and lateral hypothalamus observed in the present studies are quite suggestive that these forebrain and rhinencephalic structures have a varying influence over the release of various gonadotropins like FSH, LH and LTH from the anterior pituitary. There is so much of intermingling of the fibers in the circuitous neural organisation of limbic forebrain structures (17) that it is very difficult to pin point or localize the lesions to an extent that it blocks or releases only one or the other secretion. These neural substrates

show a dual control—facilitatory and inhibitory, influencing the release of different gonadotropins in varying proportions and combinations. Under normal conditions this dual regulatory control perhaps serves to maintain a balanced secretion of gonadotropins and thus preserve the structural and functional integrity of the reproductive tract. This supposed balance can be upset experimentally either by destruction or stimulation of various limbic forebrain structures resultlng in, amongst other things, a variety of changes in the genital tract

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