EFFECT OF HIPPOCAMPAL LESION ON ESTRUS CYCLE AND ESTRUS ACTIVITY IN RATS

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Summary: Dorsal hippocampal lesions in adult female rats leads to alteration in the estrus cycle with preponderance of estrus phase. The activity of the animals after the lesion increases, but it remains coupled with post lesion estrus cycle.

Key words: hippocampus estrus cycle estrus activity estrogen

INTRODUCTION

Evidence demonstrating the influence of diencephalic and limbic structures on the estrus cycle of rat is well known. (1,2,7). Constant vaginal estrus has been shown to result from lesions of anterior hypothalamus and suprachiasmatic regions (2,7). Lesions in other areas like basal tuberal region of the median eminence lead to constant diestrus (8).

Preponderance of estrus has also been demonstrated after lesions of medial septum, anteromedial amygdala, head of the caudate nucleus and dorsomedial thalamus (1). Lesions in areas like basal tuberal region of the median eminence, area olfactorius, lateral amygdala and nucleus accumbens favour diestrus (1).

It is also suggested that hippocampus may be an important structure in the regulation of estrogen and progesterone secretions (7). Stimulation of hippocampus is reported to prevent spontaneous ovulation in rats (9). Lesions of hippocampus result in increased general activity of the animals (3). Simulataneous studies of the effects of hippocampal lesion on female estrus cycle and activity are lacking. Hence the present work was planned to study the effect of hippocampal lesion on estrus cycle along with the estrus activity in rats.

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MATERIALS AND METHODS

Adult female rats weighing 150-200 *g* and showing 4-5 days estrus cycle were selected for the study. Each rat was housed individually in a temperature controlled room (25°C ± 1) in separate cages with 10 hr of light (8.00 hr to 18.00 hr). They were allowed water and rat diet *ad lib.*

1. Study of estrus cycle by vaginal smear technique :

Vaginal smears from each rat were studied daily by obtaining vaginal secretions with the help of saline swabs. Smears were examined after staining with methylene blue.

2. Measurement of activity :

An activity cage (30 cm x 30 cm x 30 cm) was made in the form of light weight cubicle with transparent side walls. The cubicle was suspended by its ceiling to an isometric lever, which recorded on the smoked paper attached to a slow moving drum. Any movement of the animal placed in the cage brought lever movement, which was recorded in the form of a curve on the drum. When the animal is inactive, the lever traces a base line above which different levels of activity can be recorded.

In the beginning, 24 hr continuous activity was recorded in some rats. But subsequently, recordings were restricted to at least six hours of the day and that too on the fixed hours of the morning and evening.

Total period during which the animal was active during six hours of activity recordings was estimated. The duration of activities on the different days of estrus cycle were then used for quantitative comparisons (Fig. 2).

The activity was recorded for atleast three normal cycles before the hippocampal lesions. After the lesions, recordings were continued till the animal was sacrificed or till the cycle became regular.

3. *Surgical procedure :*

Suction technique used by Kim *et al.* (3) in their studies, was also used by us for hippocampal lesion. This method was considered better, as it was thought desirable to produce lesions of large size in dorsal hippocampus. Since the ablation of overlying cortex is unavoidable, a group of rats were subjected to cortical lesion only to see the effects of such cortical lesion. This procedure is less likely to result in irritative foci as observed with electrolytic lesions using metal electrodes (5,6).

The animals were anaesthetised with sodium pentobarbitol (3.5 – 4 *mg*/100 *g* ip). With its head held level in a stereotaxic instrument (INCO), an opening with a diameter

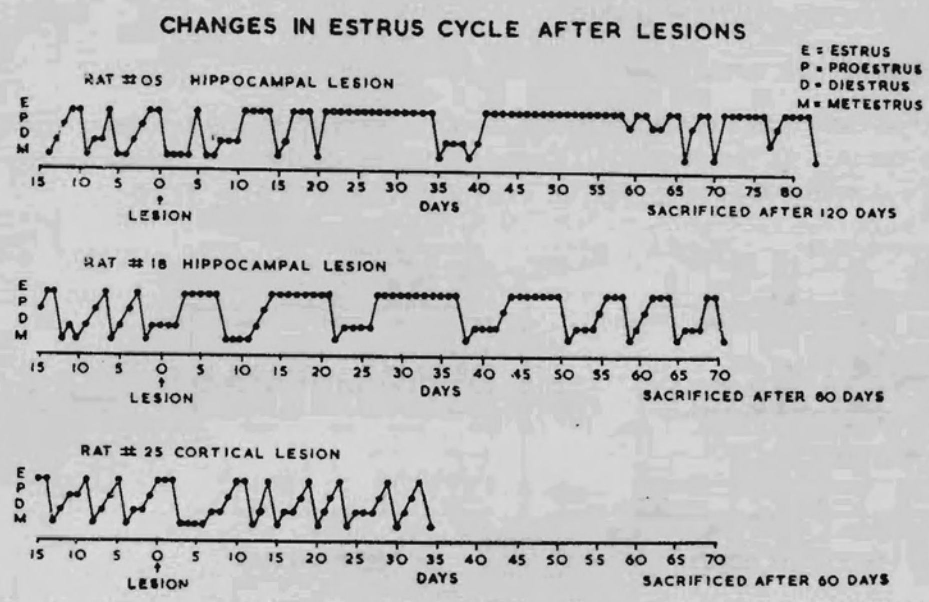

Fig. 1 : Preponderance of estrus is seen in rats after hippocampal lesion. (Upper graphs). Irregular cycles for short duration is seen in rats after cortical lesion. (Lower graph).

of around 3 mm was made in each parietal bone about 3.5mm lateral to the sagittal suture and 3.5 mm rostral to the lambda suture. The neocortical tissue was scooped on each side to expose the hippocampus which was sucked on each side with a suction apparatus using 21 gauge suction tips (3). Five animals were subjected to the same surgical procedure for cortical ablation only. Sham operated animals underwent similar surgical manoeuvres as the experimental group, without cortical or hippocampal suction.

At the conclusion of the study, the animal was sacrificed. Intracardiac normal saline followed by 10% formaline were immediately administered to perfuse the brain.

The location of the lesion was confirmed from the coronal sections of the brain (20u), stained with haematoxyline and eosin with the help of stereotaxic atlas (4). The extent of lesion was determined by plotting the lesion on the drawings of the coronal and sagittal sections of the atlas and then estimating the area of the lesion. Uterus was removed and weighed. Ovaries were examined histologically for any abnormalities.

RESULTS

Only those animals (18 in number) in which the lesions were localised in the hippocampus are included in this study.

A. Changes in the estrus cycle:

All animals showed postoperatively, irregular estrus cycles followed by preponderance of estrus phase. At the end of 8-12 weeks, in most of the rats, the cycles returned to normal pre-operative regularity (Fig. 1). Estrus Index was 2.5 ± 1.24

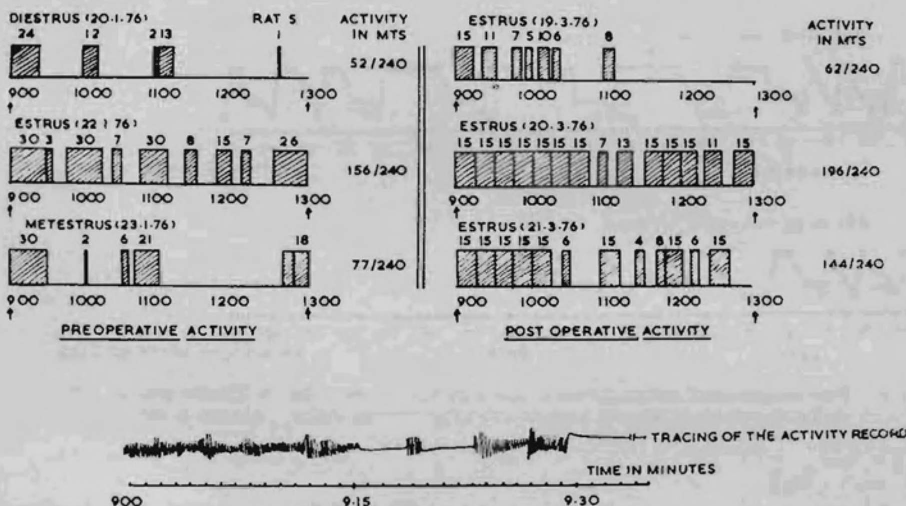

Fig. 2 : Activity of a rat recorded during identical periods (9-13 hrs) is shown before and after the lesion. Each hatched rectangle represents activity period in minutes. (Figure above the rectangle). Total duration of rats' activity increased after lesion. Pro-estrus phase is omitted in the preoperative cycle. Tracing of the activity record is shown at the bottom.

Rats with cortical ablation also showed irregular cycles immediately after the lesion, but the regularity of the cycles returned within a week or two after the surgery.

B. Changes in activity:

The activity recorded during the normal estrus cycle revealed that the rat was maximally active during estrus phase. The maximum activity was noted from the total duration during which the animal was active. In estrus, the duration of activity was long with short rest pauses. In diestrus, the rat was inactive for most of the time, the rest period being interrupted by short spells of activity. A striking correlation was also noted between the periods of maximum motor activity and the period of maximum sexual estrus behaviour when mating is most likely to occur.

When the activity in different rats was compared it was seen that the total duration during which the rat was active varied in each animal and also during various phases of the estrus cycle. But the maximum activity in each case always coincided with the estrus phase as seen in the vaginal cytology.

After the hippocampal lesions, the cyclical nature of the animal's activity was lost. The activity of long durations (maximum) correlated with estrus phase seen from vaginal smear. The activity of animal thus moved in the same direction as the phase of the estrus cycle (Fig. 2).

Such continuous and prolonged activity was not recorded in the rats with cortical ablation only.

C. *Extent of hippocampal lesions :*

In this series, the successful lesions were confined to the dorsal hippocampus (Fig. 3). The amount of tissue removed varied from 25-60%. In no case, the suction

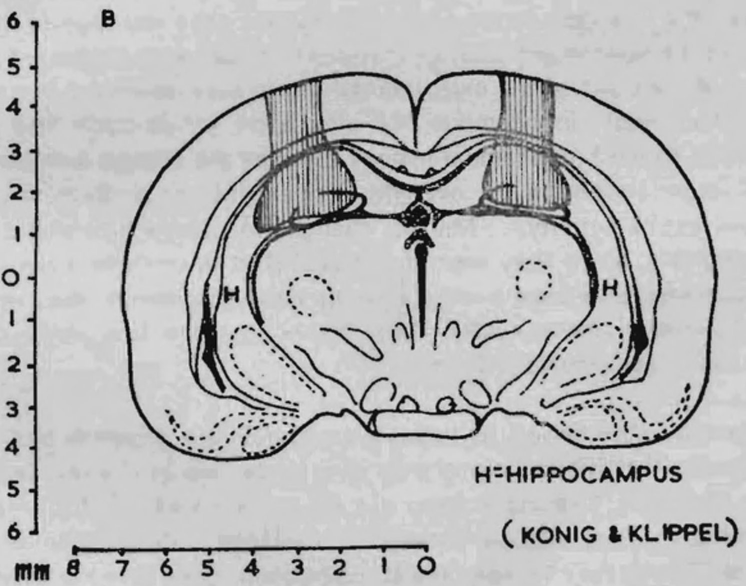

Fig. 3 : Shows symmetrical bilateral lesion of the dorsal hippocampus on schematic coronal section. A-P coordinate 2970 u according to Konig and Klippels' Atlas.

could remove the ventral hippocampal tissue. In majority of the animals the lesions on the two sides were asymmetrical. The maximum depth to which a lesion could be traced was 3 mm subcortically. The ablation was limited to 2 mm anteroposteriorly.

These variations in lesions did not materially affect the results described above which remained consistently similar in each animal. In the controls, the extent of bilateral cortical ablation overlying hippocampus was little greater than that seen during suction of hippocampus.

Uterine weights in the experimental group (115 $mg \pm 3.15$) did not vary significantly from those of control animals (130 $g \pm 5.90$). Ovarian tissue from the rats of experimental series showed few follicles and normal corpora lutea. Five animals sacrified during the course of postlesion estrus cycles showed enlarged ovaries containing large follicles with distended uteri (368 $mg \pm 7.8$).

DISCUSSION

Bilateral destruction of the cerebral cortical area does not lead to prolonged estrus. This shows that the changes in experimental animals were not due to cortical damage. In this study, dorsal hippocampal ablation resulted in cycles with prolonged estrus in female rats. Although the lesions of the experimental group also destroyed the overlying cortex in addition to the dorsal hippocampus, the effects on estrus cycle and estrus activity are essentially due to the hippocampal lesions because the control animals in which there were essential large lesions in the overlying cortex did not produce any change in the estrus cycle and estrus activity. Marked changes in uterine size and ovaries were not seen in these animals, since they were sacrificed after the regular cycles were restored. Whereas enlarged uteri and large ovarian follicles were observed in lesioned rats sacrificed in the course of persistent estrus cycles. These findings suggest that estrogen levels may be raised by ovarian sources postoperatively.

Hippocampal stimulation in mammals (7) favours progesterone secretion. Our work also indicates that hippocampus may play some role in the regulation of pituitary gonadotrophin secretion. Lesions of some of the forebrain areas (1,2,7,) are also known to produce continuous estrus studied by vaginal cytology. Restoration of normal cycles in most of these animals may be as a result of compensatory functional changes in other normal areas influencing the hypothalamus. Increased activity of these animals supports the findings (3) that hippocampus normally inhibits motor activity. High estrogen levels may also enhance the functional efficiency of neuronal circuits responsible for cyclical motor activity.

This study suggests that the dorsal hippocampus may be one of the gonadotrophin control mechanisms of the limbic system.

REFERENCES

1. Dua-Sharma, S. and K.N. Sharma. Forebrain regulation of ovarian cycle in rats: Evidence for a dual control. *Ind. J. Physiol. Pharmac.*, **17** : 17-29, 1973.

2. Flerko, B. Control of gonadotrophin secretion in the female. In Neuroendocrinology, Vol. 1, edited by L. Martins and W.F. Ganong, New York, Academic Press, pp 646-653, 1966.

3. Kim, C., H. Choi, J.K. Kim, H.K. Chang, R.S. Park and I.Y. Kang. General behavioral activity and its component patterns in hippocampectized rats. *Brain Research*, **19** : 379-394, 1970.

4. Konig, J.F.R. and R.A. Klippel. The rat brain: a stereotaxic atlas of the forebrain and lower parts of the brain stem, Baltimore, Williams & Williams Co, 1963.

5. Liberson, W.T. and K. Akert. Hippocampal seizure states in guinea pigs. *Electroenceph. Clin. Neurophysiol.*, **7** : 211-222, 1955.

6. Maclean, P.D. and J.M.R. Delgado. Electrical and chemical stimulation of fronto-temporal portion of limbic system in waking animal. *Electroenceph. Clin. Neurophysiol.*, **5** : 91-100, 1953.

7. Szentagothai, J., B. Flerko, B. Mess and B. Halasz. Hypothalmic control of the anterior pituitary – an experimental morphological study. Budapest, Akademiai Kiado, p. 301, 1968.

8. Taleisnik, S. and S.M.McCann. Effect of hypothalamic lesions on the secretion and storage of hypophysial luteinizing hormone. *Endocrinology*, **68** : 263-272, 1961.

9. Velasco, M.E. and S. Taleisnik. Effect of hippocampal stimulation on the release of gonadotrophin. *Endocrinology*, **85** : 1154-1159, 1969.