EFFECTS OF MESENCEPHALIC LESION ON THE HISTOMORPHOLOGY OF TESTIS AND SPERMATOGENESIS

M. ABID ALI*

Upgraded Department of Physiology,
Institute of Medical Sciences,
Osmania Medical College, Hyderabad-500 001

(Received on December 6, 1984)

Summary : Mesencephalic reticular formation lesions were produced bilaterally by using two epoxy-coated stainless steel electrodes. Electrolytic lesions resulted in atrophy of testes, and decreased spermatogenesis. Seminiferous tubules of lesioned rats were characterised by a general decrease in the number of cells from different generation of germinal epithelium, empty spaces, degeneration of spermatogonia, degeneration of spermatocytes I and of young spermatids. There were significant reductions in weights of the testes ($P<0.01$). Similarly the areas of cross-sections of seminiferous tubules were significantly reduced ($P<0.05$). Another note-worthy feature was a gross reduction in the complete cross section count of interstitial cells. The study strongly suggests that the mesencephalic reticular formation influences the testes and spermatogenesis.

Key words : mesencephalic reticular formation electrolytic lesion seminiferous tubules testis spermatogenesis

INTRODUCTION

A number of investigations reported in literature supported the general assumption that reticular formation can control the activity of endocrine glands, particularly the adrenals and the ovaries, (3-5, 8-11, 15-23) via the hypothalmo-hypophyseal axis in rats.

From the anatomical point of view it has been shown that the midbrain is linked to the hypothalamus by several pathways namely the medial forebrain bundle, the

*Present Address : Department of Human Physiology, Ahmadu Bello University, Zaria, Nigeria.

dorsal longitudinal fasciculus and the mammillary peduncle (3-5, 7, 20-23). Mesencephalic reticular formation activity has been shown to result both in facilitation or inhibition of gonadotrophin secretion in the female rats, (5, 20, 22, 23). It has also been demonstrated that discrete lesions in areas of mesencephalic reticular formation influence ovarian function, female reproductive behaviour, (4, 5, 12, 17-19, 21). However, the influence of similar lesions on male reproductive tract, particularly the testis, has not yet been documented.

Therefore it has been considered worthwhile to investigate and clarify the effects, if any, of discrete lesions of mesencephalic reticular formation on the testicular structure.

MATERIAL AND METHODS

Experiments were performed on male adult rats, Wistar strain weighing between 200-230 g. The animals were maintained on rat feed and water ad libitum throughout the period of study.

The animals were randomly divided into two groups of ten rats each as follows :

GROUP I Sham-operated rats which served as control.

GROUP II Experimental rats in which lesions were produced.

Surgical Technique :

After anaesthesizing the animals with phenobarbitone sodium (30 *mg/kg*, ip) two holes were drilled bilaterally in the skull and the electrodes were introduced stereotaxically into the brain. Electrolytic lesions were produced bilaterally in the mesencephalic reticular formation using two epoxy-coated stainless steel electrodes of 0.4 mm diameter and a bared tip of 0.2 mm. A current of 5 mA was delivered for 20 second to produce a discrete lesion of about 1.5 mm diameter. The sterotaxic coordinates were : Anterior-Posterior = 2.18 mm; Lateral = 1.1 mm; Ventral = 0.8 mm (vide atlas of Konig and Klippel, (13). After producing the lesions, electrodes were removed and the skin sutured. In controlled (Sham-operated) rats, electrodes were withdrawn without passing any current.

At the conclusion of the experiment after 30 days the Sham-operated and the lesioned rats were weighed and underwent vascular perfusion to fix the tissues in the vital state. The testes were removed, dried on a filter paper and weighed using Mettler electrical balance. In order to see whether changes were reflected in other organs, the kidneys were also removed for study. The testes were fixed overnight in Bouins' fixative and processed to obtain paraffin sections 4 and 6 μm thick, which were stained with Masson trichromic dye for histological and histometric studies. In order to find out the precise location of the lesions in the brains histological studies of the brains were made using 10% formaline as fixative and thionine and neutral red as stains.

Histometry :

Using standard micrometric techniques the following parameters were studied :

1. Size of seminiferous tubules, expressed as their computed area.
2. Density of seminiferous tubules expressed as the number of tubules per unit area (mm^2).
3. The distribution of tubules was estimated according to their size and density of sperm population
4. *Average complete cross section count of the interstitial cells :* All the testers were sectioned at right angles to their longitudnal axis at the widest point and a complete cross-section count of interstitial cells was made in both the Sham-operated and lesioned rats, (2, 14).

Statistical Analysis :

Statistical analysis was performed using student's t-test for paired means. The Sham-operated rats were compared with lesioned animals.

RESULTS

All the rats in groups I (Sham-operated) and II (lesioned rats) showed prompt recovery within 2-3 hours after the operation. Some of the animals in group II showed hyperactivity for varying periods (4-14 days) with marked gnawing movements. There were no significant changes in body weight and food intake of lesioned rats.

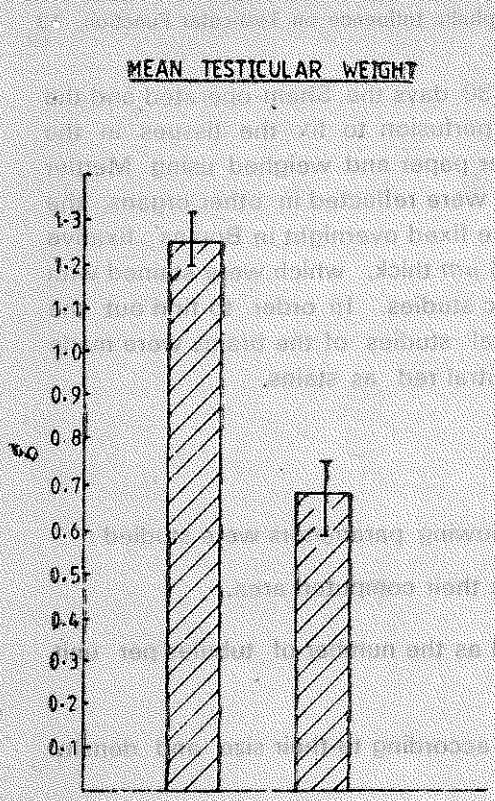


Fig. 1 : Influence of mesencephalic lesion on testicular weight of albino rats.

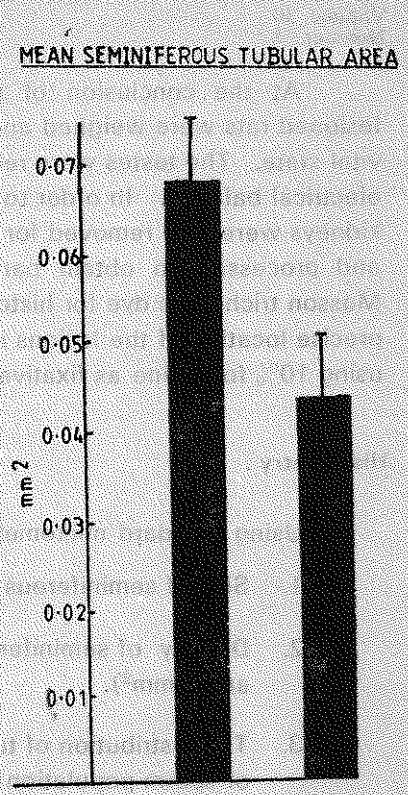


Fig. 2 : Influence of mesencephalic lesion on seminiferous tubular area of albino rats.

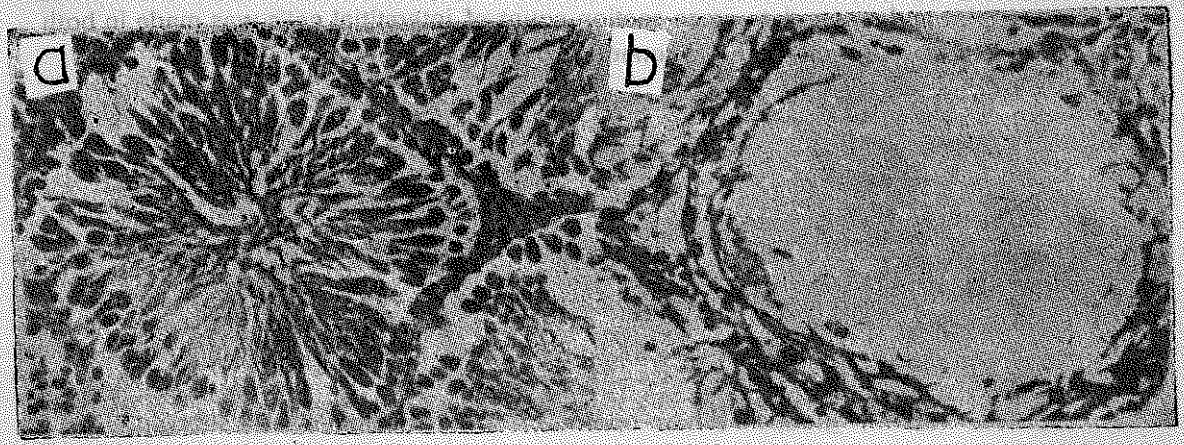


Fig. 3 a & b : Testes fixed in Bouins' fixative and stained with Masson trichromic dye. Magnification × 400. Cross section of seminiferous tubules from :

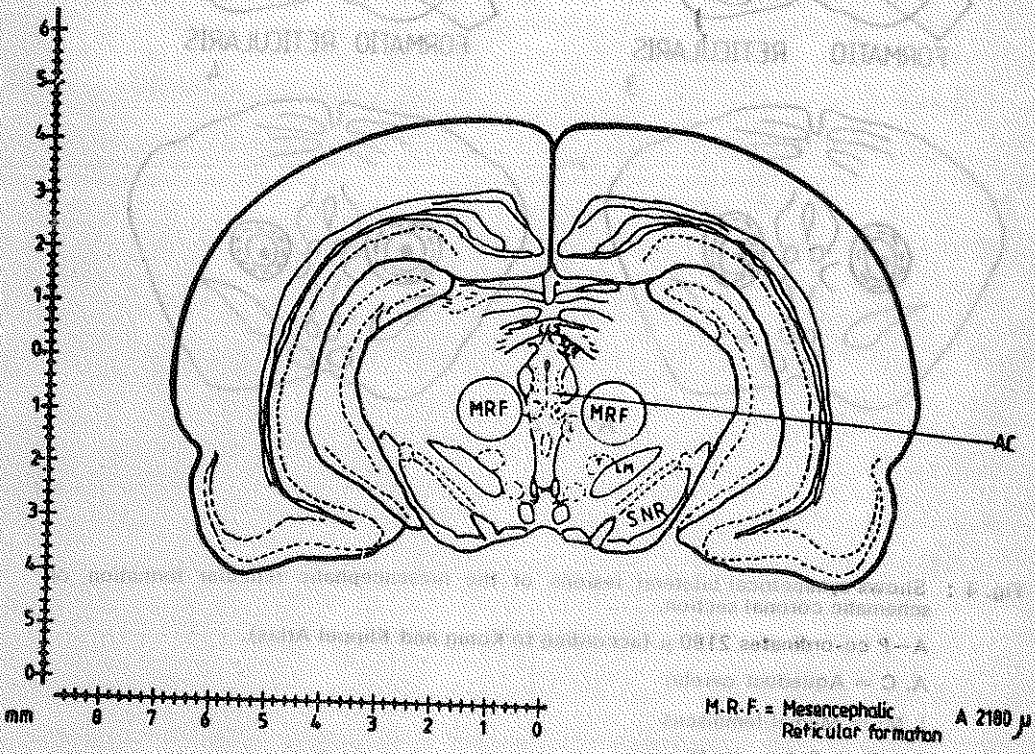
(a) Sham-operated rats autopsied at 30 days, showing healthy germinal epithelium and high population of spermatozoa.

(b) Lesioned rats autopsied at 30 days, showing degeneration of germinal epithelium with absence of spermatozoa.

The weight of testes was significantly decreased ($P < 0.01$) in lesioned rats when compared to testicular weight in sham-operated animals (Fig. 1).

The diameter of seminiferous tubules and their computed areas in lesioned animals were significantly decreased ($P < 0.05$) (Fig. 2). The number of seminiferous tubules per unit of lesioned rats was significantly increased ($P < 0.01$) as compared to Sham-operated animals.

The lesioned rats showed depressed spermatogenesis as demonstrated by a general decrease in the number of cells from different generation of germinal epithelium, empty spaces, degeneration of spermatogonia (hyperstained nuclei and nuclei with large vacuoles) and degeneration of young spermatids in comparison with those of control rats which had healthy germinal epithelium and high population of spermatozoa (Fig. 3a and b). The average complete cross section count of cells was significantly reduced ($P < 0.01$) in lesioned animals as compared to sham-operated rats. Tables I and II show body weight and testicular weights of group I and II rats with histometric data.



The approximate location of the lesion area in discrete brain regions of group II rats on frontal planes of anterior posterior level is shown in Fig. 4 using the stereotaxic atlas, of Konig and Klippel (13).

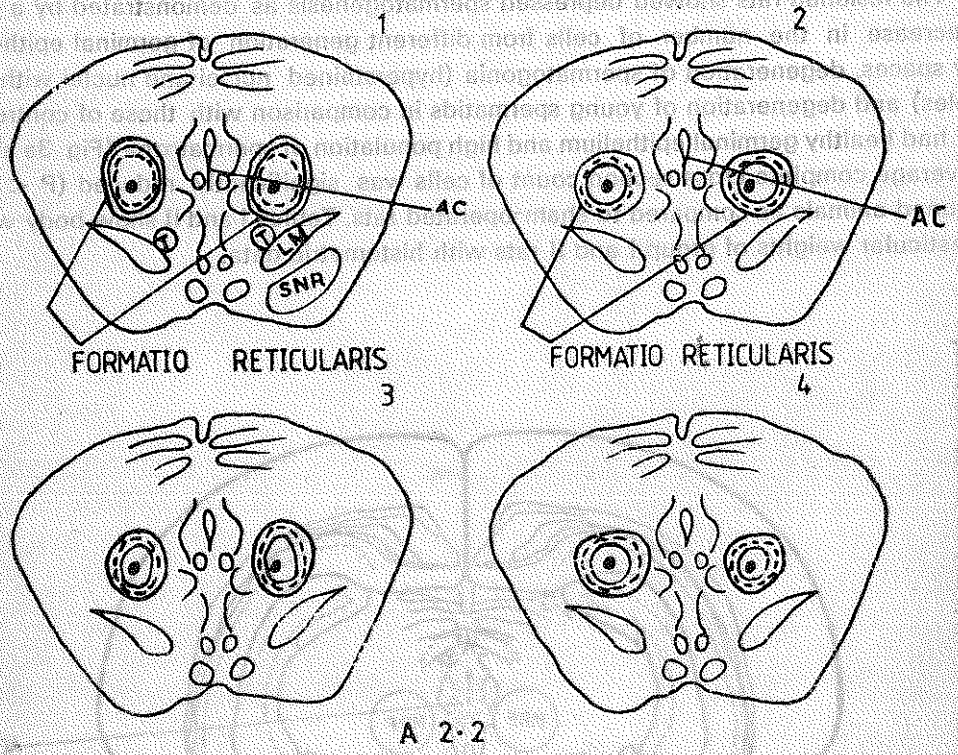


Fig. 4 : Shows symmetrical bilateral lesions of the mesencephalic reticular formation on schematic coronal section.

A—P co-ordinates 2180 μ (according to Konig and Klippel Atlas).

A. C = Aqueduct cerebri

L M. = Medial lemniscus

S N R. = Substantia Nigra.

TABLE I : Mesencephalic influence on testicular histomorphology.

	Body Weight (g)	*Testicular Weight (g)*	*Tubular Area (mm^2)*	*No. of Tubules per sq. mm*	*Distribution of Tubules according to sperm density*		
					High (above 200)	*Medium (100—200)*	*Low (below 100)*
Sham-operated rats (Group I)	206.25 ±7.98	1.250 ±0.06	0.067 ±0.008	18.60 ±0.45	61.50 ±1.85	29.13 ±2.82	9.38 ±1.52
Lesioned rats (Group II)	211.13 ±5.87	0.699* ±0.080	0.043+ ±0.007	25.19* ±0.71	2.50* ±0.05	21.00+ ±6.39	76.50* ±8.50

Tha values represent Mean ± SEM
* = P <0.01—Student t-test
+ = P <0.05—Student t-test.

TABLE II : Body weight and organ weight of 10 Sham-operated (Group I) and 10 Lesioned rats (Group II) together with histometric data.

	No. of interstitial cells per cross-section	*Kidney weight (g)*	*T.W. × 100 B.W.*
Sham-operated rats (Group I)	918.500 ±11.2422	944.000 ±23.6643	0.6060
Lesioned rats (Group II)	404.80* ±6.6965	939.00 ±23.3095	0.3310

The values represent mean ± SEM
* = P <0.01 student t-test.

DISCUSSION

The study demonstrates that bilateral electrolytic lesions in mesencephalic reticular formation affect the gonads and result in marked reduction in testicular weight, seminiferous tubular area and spermatogenesis. No appreciables changes were observed in body weight and food intake of experimental animals.

The reduction of testicular weight in lesioned rats is highly significant ($P < 0.01$) in that the normal testis which was 0.61% of the body weight was reduced to 0.33% in lesioned rats. It would thus seem that the effects were specific since the reduction in testicular weight was not reflected in other organs such as the kidneys. It is strongly suggested that this effect might have been mediated through neurohormonal pathways controlling gonadal activity (3-12, 14-22).

The importance of studying the diameters of seminiferous tubules is in accordance with the observation made earlier by Coombs and Marshall, (7) who found a distinct diminution in the diameters of seminiferous tubules within two months of hypophysectomy. The diminution in diameter of seminiferous tubules and their computed areas in the present study is highly significant ($P<0.05$), and comparable with that observed by Coombs and Marshall (7).

Sexual maturation of the male of many species of rodents is accompanied by marked changes in the hypothalamic, pituitary and gonadal hormones as well as possible changes in the sensitivity of the hypothalamus to gonadal steroidal feed back (2-12, 15-23). Changes in both type and amount of circulating androgens are also known to occur during sexual maturation of the male. These changes in circulating androgens indicate that specific changes also occur in the Leydig cells during maturation of reproductive system (10, 12, 16, 18).

Depressed spermatogenesis is clearly demonstrated in this study by reduction in testicular weight, size of seminiferous tubules and relative volume of interstitial cells. These changes suggest a decreased gonadotrophin secretion and consequently decreased androgen production and depressed spermatogenesis.

In agreement with the decreased diametres of seminiferous tubules in lesioned rats, the tubular concentration per unit area was significantly increased ($P < 0.01$). This result was not unexpected since decreased tubular diameter would consequently lead to an increased in number of tubules that can be accommodated per unit area.

A dual influence of limbic system on gonadotrophin secretion has been demonstrated (3, 5, 6, 20-23), faciliatatory influences arriving from the amygdala and inhibitory ones coming from the hippocampus. Career and Taleisnik, (5) demonstrated that electrochemical stimulation of mesencephalic structures in female rats on gonadotrophin secretion were transmitted to the hypothalamus directly via the dorsal longitudinal fasciculus or through medial fore-brain bundle-hippo campus-medial corticohypothalmic tract. Career and Taleisnik (5) also observed stimulatory effects on the release of gonadotrophins in females rats after mesencephalic stimulation in the dorsal tegmentum, lateral and inferior to aqueductal grey. Morphine, atropine and pento-barbital, adminstered to rats in doses that blocked ovulation, also elevated midbrain threshold for E.E.G activation (19). Intracerebral injections of morphine and met-enkepha-linamide in mesencephalic reticular formation had been shown to produce behavioural and analgesic effects. This also had been shown to alter hypothalamo-pituitary axis and LH secretion in male and female rats (11, 12, 19).

Sen, Singh and Saranghi (19) had observed rupture of graffian follicle, increase in ovarian weight and remarkable changes in vaginal cytology on stimulation of mesencephalic reticular formation. They suggested that mesencephalic reticular formation controls LH release from the anterior pituitary in the same way as ACTH, and intact median eminence is necessary for this effects.

Lesions produced in the present series conform in general to above area and suggest the possibilities of pathways similar to those mentioned earlier in the text. The results of this study indicate that the region, "mesencephalic reticular formation" has a facilitatory influence on hypothalamo-hypophyseal axis and in whose absence (e.g. after lesions), atrophic changes are seen in testicular morphology with decreased spermatogenesis. There is thus considerable, anatomical, electrophysiological and behavioural evidence to show that mesencephalic reticular formation influences gonadal activity and the area is reciprocally connected to the hypothalamo-hypophyseal complex (3, 6, 8-14, 16-23). As has been found in the present study, the common central hypothalamo-hypophyseal axis acts on the ovaries as well as testes.

ACKNOWLEDGEMENTS

This work forms part of a thesis submitted for the degree of Doctor of Medicine, (Physiology) at Osmania University, Hyderabad, India.

I acknowledge my profound thanks to Professors P. Simhadri and Bilquis M.A. Rasheed of the Upgraded Department of Physiology, Institute of Medical Sciences,

Osmania Medical College Hyderabad, India for their valuable guidance. I also thank Professor K.N. Sharma, Department of Physiology, University College of Medical Sciences, New Delhi—India and Dr. I.O. Adeoshun of the Department of Human Physiology, Ahmadu Bello University, Zaria for their valuable suggestions regarding the manuscript.

REFERENCES

1. Abid Ali, M., P. Simhadri and M.A.R. Bilquis. Structural changes in testis as a result of electrolytic lesion of midbrain reticular formation. *Ind. J. Physiol. Pharmac.*, **17** : 163, 1973.
2. Baillie, A.H. Biology of Leydig cells : histochemical and histological changes following high epididymal obstruction. *J. Endrocrinol.*, **20** : 339-344, 1960.
3. Barone, F., C.M.J. Wayner, I. Zarco de Coronado and W.H. Tsai. Mesencephalic reticular formation effects on hypothalmic neuronal activity. *Brain Research Bulletin*, **7** : 419-425, 1981.
4. Career, H.F. and S. Taleisnik. Neuronal pathways associated with mesencephalic inhibitory influence on gonadotrophin secretion. *Brain Research*, **38** : 299-313, 1972.
5. Career, H.F. and S. Taleisnik. Effects of mensencaphalic stimulation on the release of gonadotrophins. *J. Endocr.*, **48** : 527-537, 1970.
6. Catherine, R. and V. Wylie. Influence of Corticotrophin Releasing Factor on Reproductive function in the Rat. *Endocr.*, **114** : 914-921, 1984.
7. Coombs, C.F.J. and A.J. Marshall. The effects of hypophysectomy on internal rhythm in birds and mammals. *J. Endocr.*, **13** : 107-111, 1956.
8. Cowan, W. M., E.W. Guillery and T.P.S. Powell. The origin of mamillary peduncle and other hypothalamic connections from the midbrain. *J. Anat. (Lond.)* **98** : 345-363, 1964.
9. Critchlow, V. Blockade of ovulation in the rat by mensencephalic lesion. *Endocr.*, **63** : 566-610, 1958.
10. Ghandran, R., J.G. Lewis. and G.D. Chisolm. Serum testosterone and dihydro-testosterone changes with ages in the rat. *Steroids*, **25** : 753-762, 1975.
11. Henry, J.H. and D.D. Spring. A comparison of analgesic and behavioural effects of (D—Ala) Met—Encephalinamide and morphine in the mesencephalic reticular formation of rats. *Life Sciences*, **23** : 1229-1240, 1978.
12. Kalra, S.P. Neural loci involved in naloxone-induced lutenizing hormone release : effects of a norepinephrine synthesis inhibitor. *Endocr.*, **109** : 1805-1810, 1981.
13. Konig, J.F.R. and R.A. Klippel. The rat brain. A stereotaxic atlas of the fore brain and lower parts of the brain stem. Baltimore : The Williams and Wilkins Co., 1963.
14. Kothari, L.K., D.K. Srivastava, Pushpa Mishra and M.K. Patni. Total Leydig cell volume and its estimation in dogs and in models of Testis. *Anat. Rec.*, **174** : 259-264, 1972.
15. Lescoat, G., D. Lescoat and D.H. Garnier. Influence of adrenalectomy on maturation of gonadotrophin function in the male rat. *J. Endocr.*, **95** : 1-6, 1982.
16. Payne, A.H., R.P. Kelch, E.P. Murno and J.T. Keralan. Hypothalamic, Pituitary and testicular function during sexual maturation of the male rat. *J. Endocr.*, **72** : 17-26, 1977.
17. Paxinon, G. and D. Bindra. Hypothalamic and midbrain neural pathways involved in eating, drinking, irritability, aggression and copulation in rats. *J. Comp. Physiol. Psychol.*, **82** : 1-14, 1973.

18. Piacsek, B.D. and M.P. Goodspeed. Maturation of pituitary gonadal system in male rat. *J. Reprod. Fertil.*, **52** : 29-35, 1978.

19. Sawyer, C.H., B.V. Critchlow and C.N. Barrclough. Mechanism of blockade of pituitary activation in the rat by morphine, atropine and barbiturates. *Endocr.*, **57** : 345-350, 1955.

20. Sen, R.N., B Singh and N.N. Saranghi. The pituitary gonadotrophic releasing activity of midbrain reticular formation. *Ind. J. Physiol. Pharmac.*, **12** : 34, 1967.

21. Taleisnik, S., M.E. Velasco and J.J. Astrada. Effects of hypothalamic differentiation in the control of lutenizing hormone secretion. *J. Endocr.*, **46** : 1-7, 1970.

22. Velasco, M.E. and S. Taleisnik. Release of gonadotrophins induced by amygdaloid stimulation in the rat. *Endocr.*, **84** : 132-139, 1969a.

23. Velasco, M.E. and S. Taleisnik. Effects of hippocampal stimulation on the release of gonadotrophins. *Endocr.*, **85** : 1154-1159, 1969b.