EFFECT OF AMYGDALOID LESIONS ON ESTROUS BEHAVIOR AND GONADOTROPIN SECRETION IN PEROMYSCUS MANICULATUS BAIRDII

by

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INTRODUCTION

Estrous behavior and hormonal regulation of estrous phenomena have been studied by investigators for many years. In the course of these investigations many theories have been postulated in order to explain the neuro and hormonal phenomena associated with estrous regulation in various domestic and wild animals. The first systematic study of the estrous cycle was undertaken by Stockard and Papanicolaou (1917) in a common laboratory animal, the guinea pig. Employing the vaginal smear technique, these investigators presented a detailed picture of the histological and physiological characteristics in the reproductive tract associated with estrous cycling. Several years later detailed descriptions of estrous cycles were established in the rat (Long and Evans, 1922) and mouse (Allen, 1922). Since that time a voluminous amount of literature has been published concerning interplay between environmental stimuli with gonadotropin-steriod interaction on the physiology and biochemistry associated with estrous cycling.

Prior to 1940, it was generally believed that circulating levels of estrogens and progestagens acted directly on the anterior pituitary (pars distalis or adenohypophysis) in a servo-regulatory "feedback" mechanism to regulate the various stages of the estrous cycle (Moore and Price, 1932). During the 1920's there appeared in the literature evidence that implicated yet another area of the brain (diencephalon) in estrous regulation. Baily and Brewer (1921) and Camus and Roussy (1922) working with the dog, and Smith (1927) with the rat, reported genital atrophy in animals with hypothalamic damage. Smith demonstrated that genital atrophy could be induced by damaging the median eminence

without impairment to the pituitary. In addition, clincal evidence clearly indicated that hypothalamic-hypophysial interrelationships existed in reproductive processes. Hypothalamic lesions were reported to be responsible for hypogonadism, either alone (Hecker and Werren, 1937), in conjunction with Frohlich's adiposogenital syndrome (Dott, 1938 and Smith, 1927) or the Laurence-Moon-Biedl syndrome (Ornstein,1932). Also, precocious puberal development was attributed to destruction of a posterior hypothalamic mechanism caudal to the tuber cinereum (Weinberger, 1941).

Dey and his co-workers were the first to assign specific gonad regulating functions to the hypothalamic nuclei. Experimental damage confined to the anterior hypothalamus of the guinea pig was found to elicit genital hypertrophy, continuous vaginal cornification and marked follicular development with corpora lutea (Dey et al., 1940 and Dey, 1943). These authors postulated that such animals were unable to secrete luteinizing hormone (LH). Dey (1943) also reported that lesions confined to the junction of the pituitary stalk (arcuate nucleus) produced atrophic genitalia and constant diestrus. No further work concerning the influence of the anterior hypothalamic area (AHA) on estrous regulation was conducted until Hillarp (1949) extended Dey's procedure to the rat. By making small, bilateral, superficial lesions between the paraventricular nucleus (PVH) and the hypophysial stalk, constant estrous could be produced; thus confirming Dey's original experiments.

Early electrical and chemical stimulation experiments added much to the knowledge of estrous regulation. Strong, diffused electrical stimulation applied to the brain elicited an ovulatory and pseudo-

pregnant response in the estrus rabbit (Marshall and Verney, 1936) and the rat (Harris, 1937). By placing electrodes in discrete nuclei of the rabbit hypothalamus, Harris localized several of the active structures. Among these areas were the tuber cinereum, preoptic area (POA) and posterior hypothalamus. Intravenous administration of copper (Fevold et al., 1936) and cadmium salts (Emmens, 1940) or various plant juices (Bradbury, 1944) were also known to induce ovulation in the rabbit. However, only 1/200 to 1/300 of the intravenous dose of copper acetate was needed to induce ovulation if injected into the region of the third ventricle. Since there are no demonstrable nerve endings terminating in the anterior pituitary from the hypothalamus, in mammals, Green and Harris (1947) and Harris (1948) concluded that the neural factors which control the adenophypophysis are humorally transmitted from the hypothalamus and neurohypophysis to the pars distalis via the hypophysial portal system.

More recent investigations into this area are concerned with (1) quantitating the effects of lesions and stimulation on the above mentioned nuclei, (2) determining the neuro and neurohumoral pathways leading to these nuclei from various parts of the brain and (3) quantitating the pituitary and serum levels of gonadotropins as a result of lesions or stimulation of the various hypothalamic nuclei.

Szentagothai et al. (1962) and Flerko (1963) established the importance of the "hypophyseotropic" area (lateral and ventral hypothalamus) in the hypothalamus by demonstrating the formation of acid-Schiff positive cells and "castration cells" in pituitary grafts situated in an area, extending from the paraventricular nuclei downward

to the optic chiasm on to the rear as far as the mammillary region, in gonadectomized rats, whereas pituitary grafts in other areas of the brain failed to produce this effect. By implanting grafts of various other tissues into the "hypophyseotropic" area of hypophysectomized rats, Flerko and Szentagothai (1957) established that pituitary grafts were the only tissue able to induce recycling. Meanwhile, experiments involving electrical stimulation of the "hypophyseotropic" area added convincing evidence of its importance in estrous regulation.

Electrical stimulation of the "hypophyseotropic" area elicited the ovulatory response (Harris, 1937 and Saul and Sawyer, 1957) whereas lesions in the middle portion of this area inhibited ovulation induced by copulation in the rabbit even in the absence of ovarian atrophy (Sawyer, 1959). Critchlow (1958) demonstrated the importance of this area in LH regulation by electrically stimulating the tuber cinereum of barbiturate blocked rabbits to release enough LH to induce ovulation. From these experiments it was concluded that the "hypophyseotropic" area is of vital importance in gonadotropin secretion from the pituitary.

The effects of lesions in the AHA in adult female rats appear to result in an increased production and release of follicle stimulating hormone (FSH) (Bogdanove and Schoen, 1959 and van der Werff ten Bosch, 1959). In adult female rats estrogen was effective in retarding hypersecretion of FSH after castration, only if the paraventricular nuclei were intact (Flerko, 1959), while local application of estrogen to this region by ovarian autotransplantation results in a decreased FSH response as evidenced by lowered uterine weights (Flerko and Szentagothai, 1957). This suggested that a negative feedback mechanism

existed in the anterior hypothalamic area. Therefore, it seems, at least in the female, that lesions in the anterior hypothalamic area result in augmented FSH production, and infers that the anterior hypothalamic area is an inhibitory center for the production or release of FSH.

Other lesioning experiments involving this area established that LH regulation was also impaired. The pituitaries of anterior hypothalamic, preoptic or superchiasmic area lesioned rats are able to produce LH (Barraclough and Gorski, 1961), and under certain circumstances (castration) release it in sufficient quantaties to induce ovulation (Flerko and Bardos, 1961), but fail to do so under the lack of such stimuli (van der Werff ten Bosch, 1962). As a result, the increase in systemic estrogen creates a constant stimulus for LH release by the pituitary. This in effect depletes the pituitary of its LH stores and ovulation cannot occur even when the animal is stimulated. This phenomena was realized in experiments with androgen-sterilized (androgenized) female rats, which exhibit a syndrome of constant vaginal estrus similar to the anterior hypothalamic or preoptic lesioned animals (Taleisnik and McCann, 1961), whose pituitaries are low in LH concentration. However, if they are first primed with progesterone, the pituitaries maintain LH levels comparable to normal proestrus rats, and furthermore, if the pituitaries of these progesterone primed rats are stimulated, ovulation will occur (Gorski and Barraclough, 1961).

Without doubt, the anterior hypothalamic area and preoptic area lesioned animals are under the influence of estrogen (Hillarp, 1949). However, Hillarp was unable to explain why circulating estrogens are

unable to inhibit gonadotropin release in these constant estrus animals. This question was later resolved in the discovery that intact rats united parabiotically to a spayed partner will elicit an increase in gonadotropin secretion due to the negative feedback effect of estrogen. Daily injections of estradiol (1.0 ug) were found to inhibit the gonadotropin release (also negative feedback), however, daily injections of estradiol to parabiotically united spayed-anterior hypothalamic lesioned animals failed to block gonadotropin release (Flerko, 1963). The reason that the animals lesioned in the AHA are unable to store gonadotropins in the presence of high levels of circulating estrogens is mainly because lesions eliminate the estrogen sensitive feedback mechanism in the anterior hypothalamic or preoptic areas.

From the above experiments Flerko (1963) and Barraclough and Gorski (1961) postulated the following functions for the hypothalamic nuclei: (1) the anterior hypothalamic and preoptic areas serve as the servoregulatory feedback receptor mechanisms for circulating levels of estrogen and (2) that two levels for gonadotropin regulation exist in the hypothalamus. The "hypophyseotropic" area is concerned with production of FSH and LH and their continuous release at a basal level, while the anterior hypothalamic and preoptic areas act as the release regulating mechanism for FSH and LH in order to maintain normal reproductive functions.

The regulation of gonadotropins, in the preceeding paragraphs, was discussed in relation to the interoceptive mechanism (steroid feedback) of the organism. However, it is well known and well documented that exteroceptive stimuli also influence reproductive

function and mating behavior. Certainlly light (Fiske, 1941), temperature and humidity (Lee, 1926), olfaction (Brooks, 1937; Bruce, 1959 and Parkes and Bruce, 1960), tactile (Denenberg, 1962) and sociological and environmental stimuli (Eleftheriou and Bronson, 1962; Bronson and Eleftheriou, 1963 and Bronson, Eleftheriou and Garick, 1964) are known to modify reproductive function. There is common agreement among investigators that these stimuli are mediated via the neocortex and certain subcortical structures to the sensitive gonadotropin regulating complex in the hypothalamus (Arvey, 1964; Flerko, 1963 and Barraclough, 1964).

Anatomically the hypothalamus is considered as part of the limbic system (Johnson, 1923; Pribram and Kouger, 1954 and Pribram, 1961). Therefore, it is connected to the basal septal region, caduate nucleus, reticular formation, amygdala, hippocampus, thalamus, subthalamus and neocortex by nerve tracts (Mason et al., 1959; Pribram, 1961 and Goddard, 1964). The limbic system has been known for some time to control emotional and certainlly reproductive behavior. A review of the literature reveals that another component of the limbic system (the amygdala) exerts an influence on normal emotional and reproductive behavior besides the hypothalamus (Mason, 1959; Pribram, 1961 and Goddard, 1964). Dysfunction of this structure may elicit reactions ranging from rage (Bard and Rioch, 1937), placidity (Adey, 1958), hypersexuality (Anand, Chhina and Dua, 1959; Green, Clemente and deGroot, 1957 and Kling et al., 1960), learning (Fuller, Rosvold and Pribram, 1957) and endocrine dysfunction (Martin, Endrozi and Gate, 1958; Knigge, 1961; Bovard and Gloor, 1961 and Mason, 1959).

Hypersexuality appears to be more severe and more diversified in the male than in the female (Green et al., 1957; Wood, 1958 and Anand et al., 1959). In a few instances this hypersexuality can be abolished by castration or by lesioning the ventromedial hypothalamic nuclei (Schreiner and Kling, 1954) or septal region (Kling et al., 1960). In carefully mapping the nuclei of the amygdaloid complex in cats, Wood (1958) noted that discrete bilateral lesions confined to the lateral nuclei of this complex are responsible for the observed hypersexuality. Although amygdaloid lesions cause a greater amount of hypersexuality in males than in females, the effects on the genital organs show the opposite effect. Amygdalectomy in the adult male rat and cat results in considerable degeneration of the testes, whereas in the female cat the ovaries remain unaffected (Greer and Yamada, 1959; Kling et al., 1960 and Yamada and Greer, 1960). In only one case has precocious development of the reproductive tract occured after lesioning the amygdala (Elwers and Critchlow, 1960). The medial portion was found to be involved.

In addition, further evidence for amygdaloid involvement in reproductive function comes from experiments involving electrical stimulation of discrete areas in the amygdaloid complex. Ovulation with increased uterine movement can be induced in the rat (Bunn and Everett, 1957) and cat (Shealy and Peele, 1957) by stimulating the medial portion of this complex. Several of the above authors suggest that the amygdaloid complex mediates behavior via the hypothalamus on the assumption that the amygdala sends a rich network of efferent neural connections to the anterior hypothalamic and preoptic areas. Wood

(1958), also has shown that fibers originating in the amygdaloid complex terminate in the lateral hypothalamic area and ventromedial hypothalamic nuclei.

In spite of the amount of information known on the effects the amygdaloid complex exerts on reproductive functions and sexual behavior, no investigation has attempted to quantitate these effects on gonadotropin synthesis and release by measuring the amount of gonadotropin in the pituitary and plasma and correlate these amounts with histological observations of the reproductive tract. Two experiments were undertaken in order to elucidate the role the amygdala plays in regulating mating behavior and gonadotropin secretion. The animal chosen for these experiments was the female deermouse, Peromyscus maniculatus bairdii. Since no stereotaxic atlas existed for this species, a stereotaxic atlas of the forebrain was constructed in order to locate internal brain structures for the accurate placement of lesions. Experiment one was to determine which nuclei of the amygdaloid complex are responsible for normal or aberrant mating behavior, while experiment two was concerned with measuring the effects of incomplete bilateral ablation of the basolateral amygdaloid nuclei on pituitary gonadotropin synthesis, storage and release.

MATERIALS AND METHODS

CONSTRUCTION OF THE STEREOTAXIC ATLAS

Initially, five adult female deermice (P. m. bairdii) weighing 19 grams were employed to determine the vertical and horizontal zero

planes. The animals were anesthetized with 1.2 mg of sodium pentobarbitol, injected intraperitoneally, and oriented in a standard rat stereotaxic apparatus (Trent Wells Jr.) (Figs. 1 and 2). Due to the extreme angle the brain made with the horizontal zero plane using this instrument, the interaural line connecting the external auditory meati was not used as the axis for rotation of the head. Instead, turned-down ear plugs (1/16") were placed in the ears and pulled down under the skull. With the head in this position (Figs. 1 and 2) the snout was freely turned upward or downward for placement of the upper incisor bar. The upper incisor bar was used to firmly support the upper jaw at a point 4.0 mm above the horizontal plane of the ear bars. The upper incisor bar then was moved anteriorly or posteriorly until the most posterior portion of the junction of the lambdoidal and sagittal sutures formed the 0,0,0 coordinate (Fig. 3).

Gross internal structure was determined by two methods. Internal structure was ascertained by dissecting an entire head in the mid-sagittal plane (Fig. 4) and measuring positions of internal structures under a stereotaxic scope. In addition, electrolytic lesions (20 uA/20 seconds) were placed at various coordinates in the brain and their position determined histologically. The animals were decapitated, their heads trimmed of skin and placed in 10 percent neutral formalin for 24 hours. The brains then were removed from the skulls, dehydrated in dioxane, embedded in paraffin, sectioned at 25 μ and stained with Cresyl Violet Blue by the method of Powers and Clark (1955).

By comparing the transverse sections of an intact brain, the mid-sagittal dissected brain and the serial transverse sections of the

lesioned brains, the angle of sectioning (A - A') was determined (Fig. 3). Representative serial sections of a transverse non-lesioned brain were chosen to be photographed and enlarged. Diagrammatic sketches were made of the right half of each photomicrograph and mounted to its respective counterpart. The combined photomicrograph-diagrammatic sketch was photographed with coordinates to make the plates for the atlas. "A" on the A - A' axis (Fig. 3) represents the horizontal-vertical coordinate at the top-most surface of the brain which intersects the vertical plane, while A' represents the bottom-most horizontal-vertical coordinate that intersects the vertical plane. The above method of illustrating the coordinates was employed because of the inability to obtain a complete series of transverse sections sectioned parallel to the vertical zero plane of the stereotaxic apparatus. By comparing the transverse photomicrograph-diagrammatic composites, carefully noting positions of nuclei and nerve tracts, with the position of lesions in the lesioned brains, two longitudinal diagrammatic sketches were constructed.

All dimensions used in the atlas are in millimeters. In addition, the contours of the main portions of fiber tracts (striped areas) were drawn in full lines, and the approximate outlines of cell groups, nuclei and subcortical areas indicated by interrupted lines.

Brains also were lesioned at various coordinates to determine the accuracy of the atlas. Corrections were made when necessary.

EFFECT OF LESIONS ON ESTROUS CYCLING AND MATING BEHAVIOR

Adult female deermice weighing 15 to 19 g were anesthetized with sodium pentobarbitol, oriented in a stereotaxic instrument and lesioned bilaterally in either the basolateral or medial amygdaloid nuclei.

Monopolar electrodes were made by coating epilation needles (Birtcher Corporation) with an ethyl acetate-plastic solution. After the plastic air-dried, about 0.5 mm of coating was removed from the tip. A large stainless steel bar, inserted in the anus, served as the indifferent electrode. Lesions were produced by electrocoagulation using an LM-3 Radio Frequency Lesion Maker (Grass Instruments Inc.) discharging 20 μA of current for 30 to 40 seconds. The resultant lesions were less than 0.5 mm in diameter.

All females were housed individually in new or descented transparent plastic cages and given water and standard laboratory rat chow ad libitum throughout the duration of the experiment. The animals were housed in a well ventilated room after the operation and placed on a light cycle of 14 hours of artifical light. Two days were allowed for recovery from the operation after which a confirmed stud was placed in the cage with the female, and the estrous cycle followed by daily vaginal smears. If more than 20 sperm per field were found in the wet vaginal smear, the male was removed and the female isolated for ten to fifteen days at which time she was killed. At the time of death these animals were examined for pregnancy and general condition of the reproductive tract. All non-mated females were killed twenty-four days from the time of the operation. The brains of all lesioned animals were removed and treated as before. Location of lesions was confirmed by histological examination.

EFFECTS OF BASOLATERAL AMYGDALOID LESIONS ON PITUITARY AND PLASMA GONADOTROPINS

Adult female deermice weighing 15 to 19 g were anesthetized with

sodium pentobarbitol and lesioned bilaterally in the basolateral nuclear group (basolateral and lateral nuclei) of the amygdala. A monopolar electrode was again used to coagulate the desired brain area employing the same technique as before except that the lesions were produced by a High Frequency Hyfricator (Birtcher Corporation) discharging 1.5 mA of current for 7.0 seconds.

The treated animals, isolated one per cage, were taken to the animal room and housed under the same conditions as in the previous experiment. Daily vaginal smears were taken of all females until they were killed at the prescribed time. Only animals with aberrant cycles (i.e. long periods of diestrus with an occasional proestrus smear) or acyclic animals were used in the sampling. Blood and pituitaries from 3 groups per period of five to seven animals per group were collected at 1, 2 and 3 weeks following the operation. At autopsy, the ovarian, pituitary and blotted uterine tissue was weighed to the nearest 0.1 mg on a Roller Smith torsion balance while body weights were recorded to the nearest 0.1 g on a standard triple beam balance. Blood was collected in heparinized syringes by entering the orbital sinus of the eye. Plasma was obtained by centrifugation at 1000 x g for fifteen minutes in a refrigerated centrifuge and stored in a freezer for later analysis. Pituitaries were removed from the animal as quickly as possible, weighed and placed in a 2.0 ml vial with 0.5 ml of 0.85% saline. The entire vial then was frozen in a cold bath of acetone and dry ice. The heads of the lesioned animals were placed in 10% neutral formalin for histological examination. Likewise, 3 groups (5 to 7 animals per group) of normal animals in proestrus, estrus and diestrus were killed and

treated as outlined for the lesioned animals.

The pooled pituitaries were homogenized in cold saline and made up to a final concentration of 0.8 mg/ml to 1.2 mg/ml. The amount of follicle stimulating hormone (FSH) in the pituitary homogenate was assayed according to the uterine weight increase method of Klinefelter, Albright and Griswold (1943) in immature mice. Groups of five receptor mice (Swiss strain) were injected subcutaneously with six injections over five days with either homogenate (0.1 ml/injection) or various doses of standard FSH (NIH-FSH-S3 ovine) in saline and killed on the sixth day. Uterine tubes were carefully dissected out intact, trimmed of fat and weighed to the nearest 0.1 mg. A standard FSH-dose response curve was constructed from the following equation: $Y = 605.322 + 274.108 (\log X)$, where Y is uterine weight (mg/100 g of body weight) and X is mg of FSH, (Fig. 16). Pituitary FSH content was calculated in each sample using the above equation and corrected to ug of FSH per mg of pituitary tissue.

Pituitary and plasma levels of luteinizing hormone (LH) were assayed by the ovarian ascorbic acid depletion method of Schaffert and Kingsley (1955) as modified by Parlow (1961) in immature rats. Receptor rats (Holtzman strain) were pretreated (made pseudopregnant) with a single subcutaneous injection of 50 International Units of Pregnant Mares Serum Gonadotropin (Equinex) in 0.1 ml of saline, followed 56 hours later by a single subcutaneous injection of 25 International Units of Human Chorionic Gonadotropin (A.P.L.). Six days later, the following doses of standard LH (NIH-LH-S5 ovine) saline, 0.5, 1.0, 5.0, 20.0 and 100.0 µg in 1.0 ml of saline or 1.0 ml of pituitary homogenate

were injected in groups of four to five primed rats intravenously in the tail vein under ether anesthesia. In addition, 0.6 ml of plasma was injected in groups of two primed rats. Four hours later the animals were killed with ether, their left ovaries removed, weighed and assayed for ascorbic acid. The ascorbic acid content of the ovary, in mg% (mg of ascorbic acid per 100 g of ovarian tissue), was determined by the following formula:

$$\text{mg\% of Ascorbic Acid} = \frac{\text{Optical Density x dilution x 100}}{\text{factor x mg of ovarian tissue}}$$

The factor was calculated from a standard ascorbic acid solution, and its units are Optical Density per µg. Pituitary and plasma LH concentrations were calculated from a standard LH dose response curve derived from the following equation: $Y = 67.656 - 27.552 \log X$, where Y is ovarian ascorbic acid in mg% and X is ug of luteinizing hormone. The values for LH then were transformed into milliunits of LH per mg of pituitary tissue or ml of plasma (Fig. 17).

RESULTS

CONSTRUCTION OF THE STEREOTAXIC ATLAS

The stereotaxic atlas of the forebrain of *P. m. bairdii* consists of 20 plates of transverse sections and 2 longitudinal parasagittal diagrammatic sketches illustrating representative brain structures, of which only the most pertinent diagrams related to this thesis are shown here (Figs. 5 - 9). These sketches best illustrate the anatomical relationship between the amygdaloid complex and the important gonado-

tropin regulating nuclei of the hypothalamus. In addition, figures 1 and 2 illustrate the position of a deermouse in the stereotaxic apparatus at the time of the operation.

EFFECT OF AMYGDALOID LESIONS ON ESTROUS CYCLING AND MATING BEHAVIOR

The results of lesioning discrete amygdaloid nuclei in the female deermouse on estrous cycling and mating behavior are presented in tabulated form in Table 1.

TABLE 1

Effects of amygdaloid lesions on estrous cycling and mating behavior in the deermouse.

Treatment	N	Estrous condition	Estrous behavior	Percent Mating 1st Estrous	Percent Mating 2nd Estrous
Intact, Normal	25	Cycling	Mating	92	8
Medial Lesions	10	Cycling	No-mating	0	0
Basolateral Lesions	24*	Cycling	Mating	62	33

* Five basolateral lesioned animals mated in diestrus.

Lesions in the medial amygdaloid nuclei resulted in cycling but no-mating, whereas lesions confined to the basolateral amygdaloid nuclei resulted in both cycling and mating. The coordinates used to produce the medial lesions were 5.0 mm (horizontal direction) : $\pm$2.3 mm (lateral direction) : -4.8 mm (vertical direction) (Fig. 3). The average area of tissue destruction in the anteroposterior direction in the brains of animals with medial amygdaloid lesions ranged from

the ventromedial portion of the hippocampus anterior to the level of the posterior limits of the dorsal and ventromedial hypothalamic nuclei (Fig. 10). Tissue destruction in the lateral direction extended from the medial border of the basolateral nuclei including the dorsal portion of the cortical amygdaloid nuclei. The average lesion was cylindrical in nature with an average height of about 1.0 mm and diameter of 0.5 mm.

Basolateral amygdaloid lesions resulting in cycling and mating (Table 1), are diagrammaticly represented in figure 11. The coordinates used to produce these lesions were the same in the horizontal and vertical direction as those of the medial amygdaloid lesions, except, $\pm$2.8 mm was employed in the lateral direction. In the anterioposterior direction, the average area of tissue destroyed in the brains of animals with basolateral amygdaloid lesions was similar to the area destroyed in the brains of animals with medial lesions. However, in the lateral direction, the area destroyed in basolateral lesioned animals extended from the medial border of the basolateral nuclei laterally to the middle portion of the lateral amygdaloid nuclei including most of the centromedial amygdaloid nuclei. The average dimensions of these lesions were similar to the medial amygdaloid lesions.

Sixty-two percent of the animals lesioned in the basolateral nuclei mated during their first estrous cycle, whereas ninty-two percent of the normal animals mated during a comparable length of time. In addition, five of the lesioned animals mated in diestrus, which was taken to indicate hypersexuality.

EFFECT OF BASOLATERAL AMYGDALOID LESIONS ON PITUITARY AND PLASMA GONADOTROPINS

Fifty-five percent of the 100 animals bilaterally lesioned in the basolateral amygdaloid nuclei were found satisfactory (failed to cycle or had unusually long periods of diestrus) for use in this experiment. The vaginal smears of the sixty non-cyclic animals resembled that of diestrus.

Location of the lesions in the basolateral complex (coordinates; 5.5 mm : ±2.8 mm; -4.8 mm) are illustrated in figure 12. The average area of tissue destruction in the anteriopesterior direction, extended from the level of the middle portion of the dorsal and ventral hypothalamic nuclei to the anterior border of the posterior hypothalamic nuclei. Laterally, the lesions extended from the middle of the basolateral amygdaloid nuclei well into the lateral amygdaloid nuclei. In addition, portions of the cortical and centromedial amygdaloid nuclei were involved. The lesions were cylindrical in nature with an average height of about 0.8 mm and diameter of 0.5 mm.

It was found that lesions this far anterior in the basolateral nuclei increased the number of non-cycling animals about 40% as compared with lesions in the posterior portion of this nucleus.

Figure 13 is a graph illustrating the effect of basolateral amygdaloid lesions on ovarian, uterine and pituitary weight. All tissue weights are expressed as mg of tissue per 100 g of body weight (mg%) to correct for variation in body weight. A linear relation exists in the ovarian weight with progression of the estrous cycle in normal unlesioned animals. From a mean value of 93.2 mg% at diestrus,

the ovarian weight increased to a mean weight of 122.1 mg% at estrus. The effect of lesions on ovarian weight resulted in a diphasic response. From a mean weight of 108.1 mg% at one week following the operation, the ovarian weight increased to 182.2 mg% at two weeks then decreased to a mean weight of 147.9 mg% three weeks following the operation. The rise in ovarian weight at two weeks was a result of luteinization of ovarian follicles (Fig. 18). Three weeks following the operation, the ovaries were still heavily luteinized with both degenerating corpora lutea and fully luteinized corpora lutea, but growing follicles also were present (Fig. 19).

Normal weights for blotted uterine tissue rose from a mean diestrus value of 123.0 mg% to 237.1 mg% at estrus. The same general trend in uterine weight also was noted in the treated animals, except the increase was less pronounced. From a mean weight of 87.0 mg% one week after the operation, the uterine weight increased to a mean weight of 164.3 mg% three weeks after lesioning, which was comparable to that of proestrus. In addition, figure 13 shows that although the pituitary weights of the animals with lesions increased with time following the operation, the increase in weight was only 11.3 percent greater than the peak value observed for proestrus (8.8 mg%).

During the estrous cycle, normal plasma levels of luteinizing hormone (LH) decreased slightly from a mean diestrus concentration of 0.82 milliunits per ml (Fig. 14) to a mean concentration of 0.78 milliunits per ml at proestrus, then increased sharply to an estrus level of 2.0 milliunits per ml. As the plasma levels of LH increased, pituitary levels of cycling animals decreased in amount from a mean

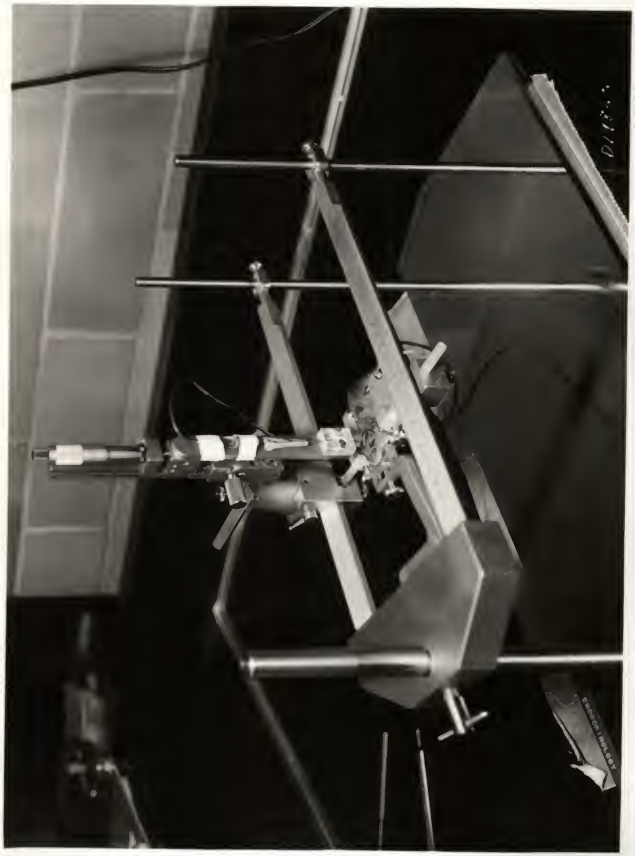
concentration of 0.6 milliunits per mg at diestrus to a mean value of 0.16 milliunits per mg at estrus.

Plasma LH levels increased linearly with time following basolateral amygdaloid lesions, while pituitary LH content decreased linearly during the same period. From a mean concentration of 0.59 milliunits per ml one week following the operation, the plasma LH content rose to a three week value of 2.62 milliunits per ml. The pituitary LH concentration at one week following the operation decreased from 0.49 milliunits per mg of tissue to a low value of 0.09 milliunits per mg three weeks following lesioning.

Pituitary follicle stimulating hormone (FSH) content in normal estrous cycling animals decreased from a mean concentration of 4.07 µg/mg of pituitary tissue at diestrus to 1.87 µg/mg at proestrus where it remained essentially unchanged into the estrus period (Fig. 15). However, a diphasic response in pituitary FSH content was noted in the treated animals following the operation. From a comparable diestrus value of 4.6 µg/mg at one week following the lesions, the pituitary FSH content increased 240% (13.95 µg/mg) at two weeks then sharply decreased to a diestrus level of 3.96 µg/mg at three weeks.

The pituitary FSH to LH ratio for normal estrous cycling females was 4.00 during diestrus, 1.79 during proestrus and 7.30 during the estrus period of the cycle.

Fig. 1. Lateral view of *P. m. bairdii* in position in a modified rat stereotaxic apparatus.



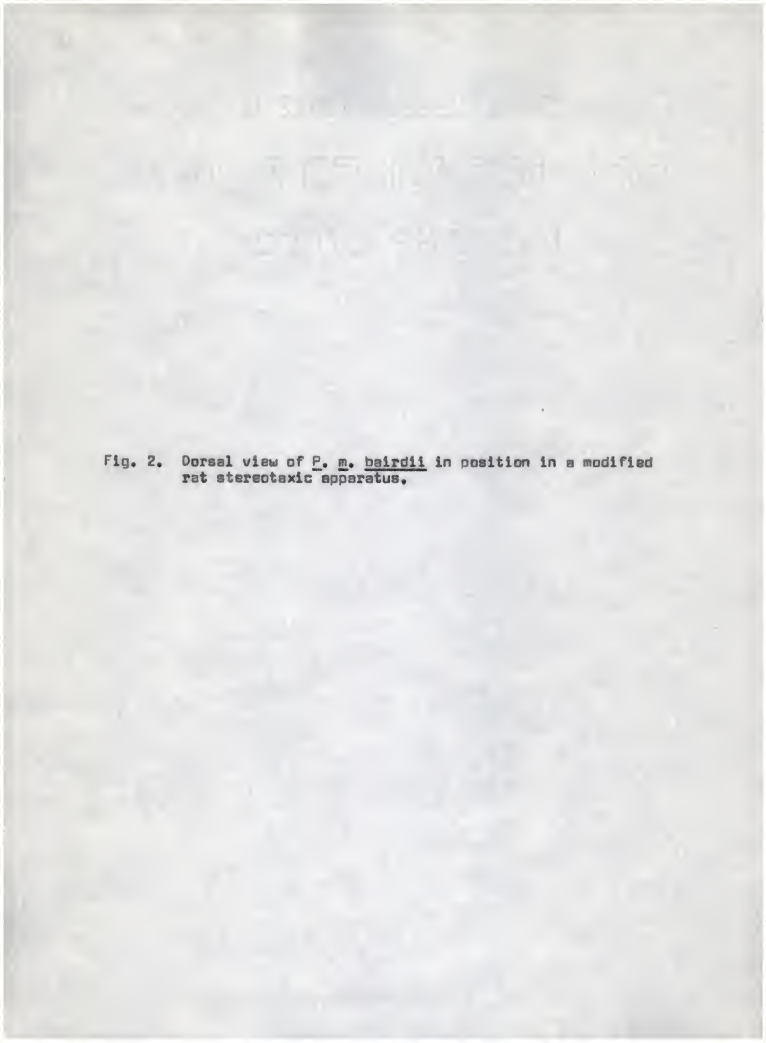


Fig. 2. Dorsal view of *P. m. bairdii* in position in a modified rat stereotaxic apparatus.

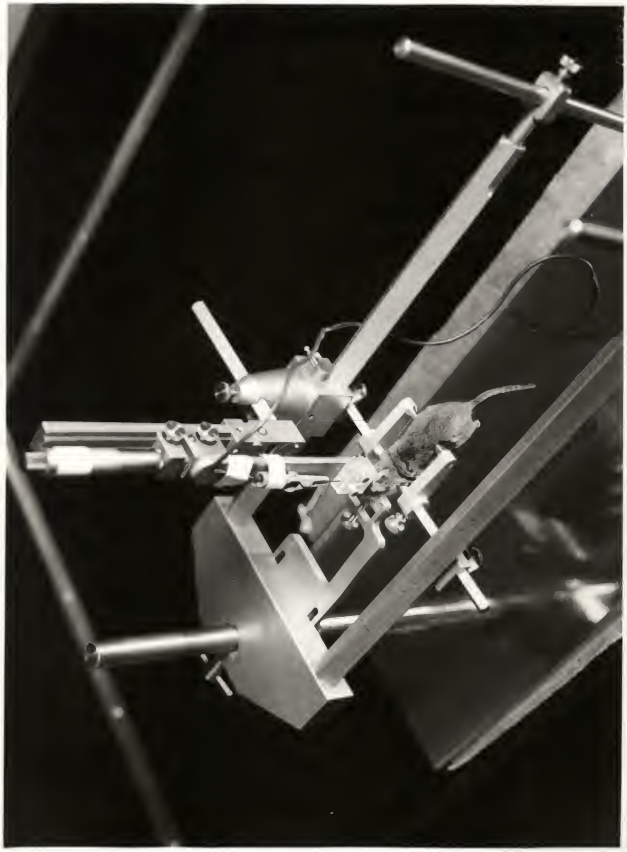
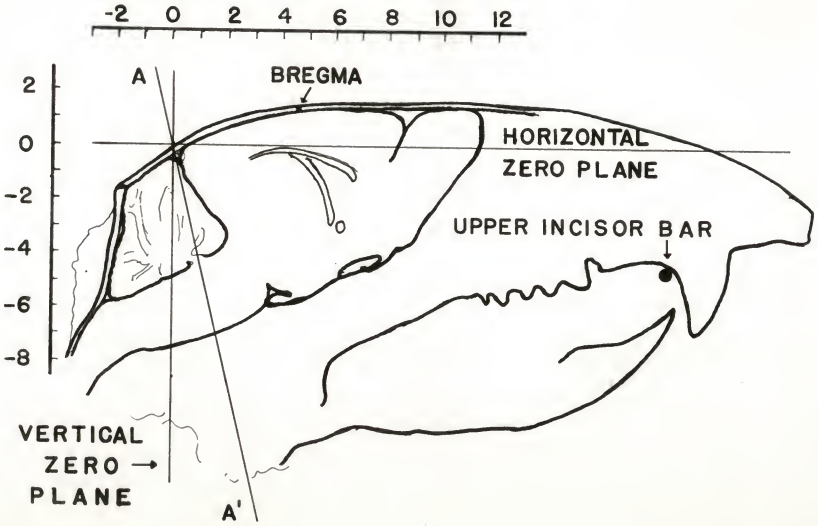


Fig. 3. Sagittal outline of skull indicating outline of brain and positions of plane at which transverse sections of brain were made.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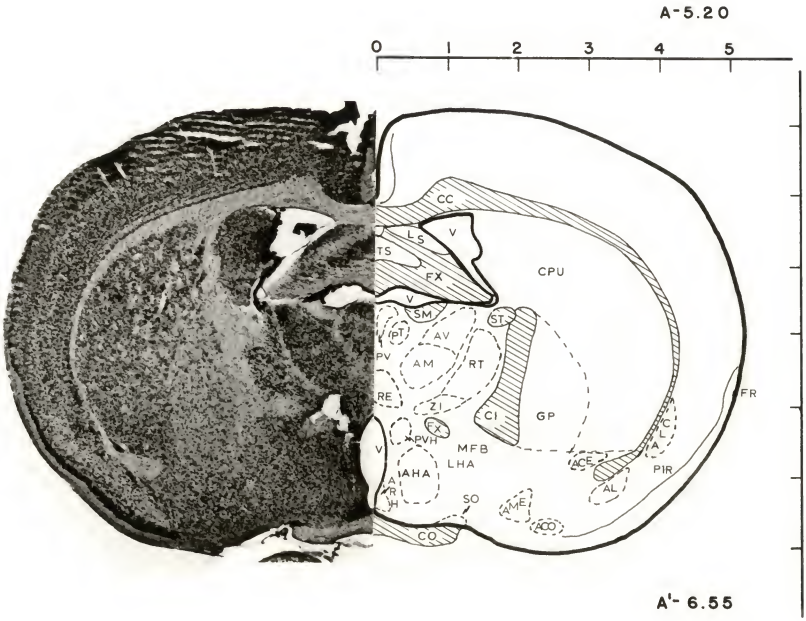
A
BREGMA
HORIZONTAL
ZERO PLANE
UPPER INCISOR BAR
VERTICAL
ZERO
PLANE
A'

Fig. 4. Sagittal section of head of *P. m. bairdii* indicating position of brain *in situ*.

Figs. 5 - 9. Composite diagrammatic-photomicrographic transverse sections illustrating representative structures in the brain of P. m. bairdii. Height and lateral scales are in millimeters. A - A' axis represents angle at which brain was sectioned. Abbreviations used to describe neuroanatomical structures:

ABL Nucleus amygdaloideus basalis pars lateralis
ACE Nucleus amygdaloideus centralis
ACO Nucleus amygdaloideus corticalis
AD Nucleus anterodorsalis thalami
AHA Area anterior hypothalami
AL Nucleus amygdaloideus lateralis
AM Nucleus anteromedialis thalami
AME Nucleus amygdaloideus medialis
ARH Nucleus arcuatus hypothalami
AV Nucleus anteroventralis thalami
CC Corpus callosum
CH Commissura hippocampi (Commissura fornicis)
CI Capsula interna
CL Nucleus subthalamicus (Luys)
CLA Claustrum
CO Chiasma opticum
CPU Nucleus caudatus/Putamen
CT Nucleus centralis tegmenti (Bechterew)
DMH Nucleus dorsomedialis hypothalami
FA Fissura amygdaloidea
FD Gyrus dentatus (Fascia dentata)
FH Fissura hippocampi

FI	Fimbria hippocampi
FR	Fissure rhinalis
FX	Fornix (Corpus, columna)
GL	Corpus geniculatum laterale
HL	Nucleus habenularis lateralis
HM	Nucleus habenularis medialis
HP	Tractus habenulo-interpeduncularis (Fasciculus retroflexus)(Meynert)
HPC	Hippocampus (Cornu Ammonis)
IP	Nucleus interpeduncularis
LHA	Area lateralis hypothalami
LS	Nucleus lateralis septi
LT	Nucleus lateralis thalami
LTP	Nucleus lateralis thalami pars posterior
MD	Nucleus mediodorsalis thalami
MFB	Fasciculus medialis telencephali (Medial forebrain bundle)
MT	Tractus mamillo-thalamicus (Vicq d'Azyr)
NTP	Nucleus posterior thalami
OA	Nucleus olfactorius anterior
OT	Tractus opticus
P	Pons
PC	Pedunculus cerebri
PF	Nucleus parafascicularis thalami
PH	Nucleus posterior hypothalami
PIR	Cortex piriformis
PMD	Nucleus premamillaris dorsalis
PMV	Nucleus premamillaris ventralis
PT	Nucleus parateanialis thalami
PV	Nucleus paraventricularis thalami
PVH	Nucleus paraventricularis hypothalami
RE	Nucleus reuniens thalami
RF	Formatio reticularis (mesencephali)
RH	Nucleus rhomboideus thalami
RT	Nucleus reticularis thalami
SM	Stria medullaris thalami
SN	Substantia nigra
SO	Nucleus supraopticus hypothalami
ST	Stria terminalis (Taenia semicircularis)
TS	Nucleus triangularis septi
TT	Tractus mamillo-tegmentalis
TUO	Tuberculum olfactorium
V	Ventriculus cerebri
VA	Nucleus ventralis thalami pars anterior
VD	Nucleus ventralis thalami pars dorsomedialis
VE	Nucleus ventralis thalami
VM	Nucleus ventralis thalami pars medialis
VMH	Nucleus ventromedialis hypothalami
ZI	Zona incerta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RE
ZI
CI
GP
FR
CX
PVH
V
MFB
LHA
AHA
A
R
H
SO
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PIR
AL
AME
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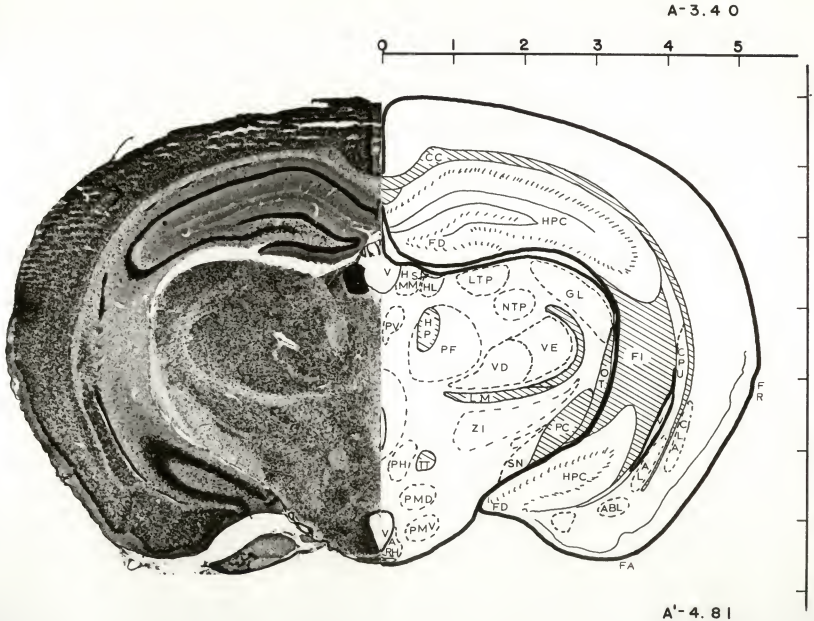
A'- 6.55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3
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1
0
-1
-2
-3
-4
-5
-6
CC
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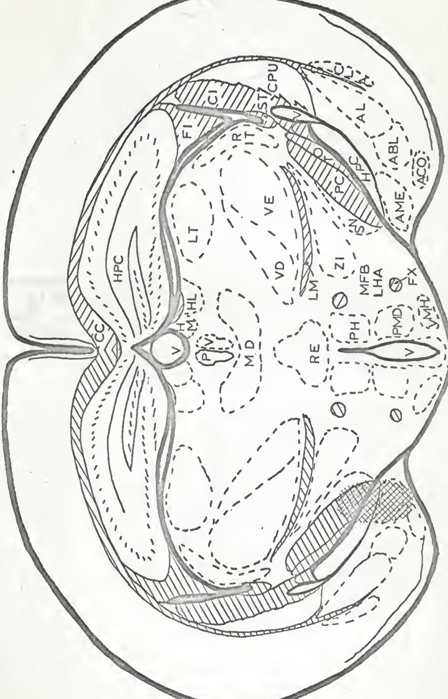
FD
V
HSD
MM
HL
LTP
GL
NTP
PV
HP
PF
VE
VD
FI
LM
ZI
PC
SN
PH
TT
PMD
PMV
VTA
RN
ABL
FA
F
R
A'-4.81

Fig. 10. Diagrammatic illustration of a transverse section of the brain of P. m. bairdii. Meshed area on left hemisphere represents location of lesions. Right hemisphere represents location of various nuclei. Coordinates of lesion are: 5.0 mm ; -2.3 mm ; -4.8 mm.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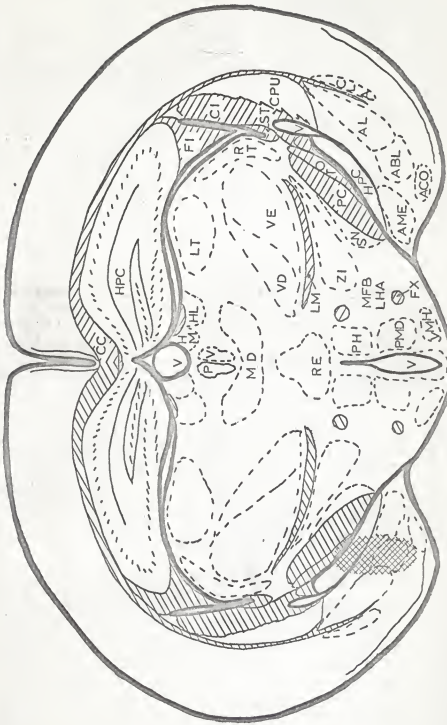
PMD
VMH
V
FI
CI
R
IT
ST
CPU
OT
PC
HPC
SN
AL
ABL
ACO
AME

Fig. 11. Diagrammatic illustration of a transverse section of the brain of P. m. bairdii. Meshed area on left hemisphere represents location of lesions. Right hemisphere represents location of various nuclei. Coordinates of lesion are: 5.0 mm : -2.8 mm : -4.8 mm.


CC
HPC
V
FI
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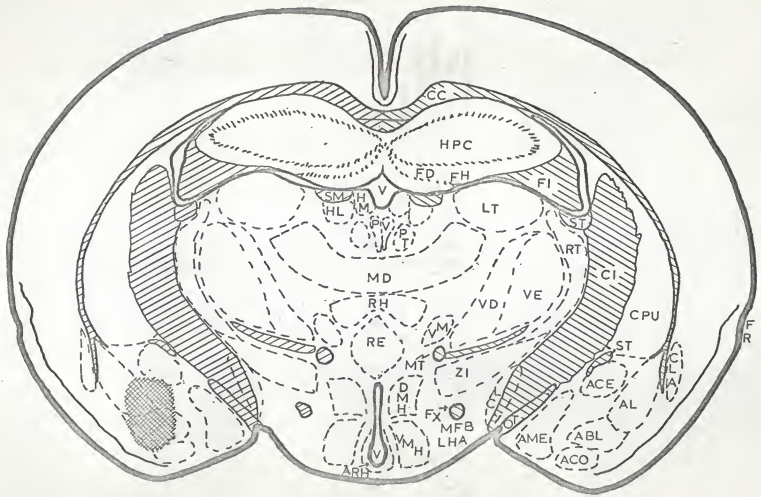
CPU
LT
M
HL
PV
MD
VE
VD
LM
RE
ZI
PH
MFB
LHA
PMD
FX
VMP
OT
AL
ABL
ACO
HPC
FC
AM
AME

Fig. 12. Diagrammatic illustration of a transverse section of the brain of P. m. bairdi. Meshed area on left hemisphere represents location of lesions. Right hemisphere represents location of various nuclei. Coordinates of lesion are: 5.5 mm ; ±2.8 mm ; -4.8 mm.


CC
HPC
FP
FH
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SM
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HL
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CI
MD
RH
VD
VE
CPU
F
R
RE
VM
ST
MT
ZI
ACE
AL
DMH
FX
MFB
LHA
AME
ABL
ACO
VM
HI
ARH

Fig. 13. Effect of basolateral amygdaloid lesions on ovarian, uterine and pituitary weights. Tissue weight is expressed as mg of tissue per 100 g of body weight (mg%) to correct for body weight variations. Stage of cycle and weeks following lesioning are plotted along the abscissa. D = diestrus, P = proestrus and E = estrus period in the estrous cycle. Each point represents the mean of three groups (5 to 7 animals/group).

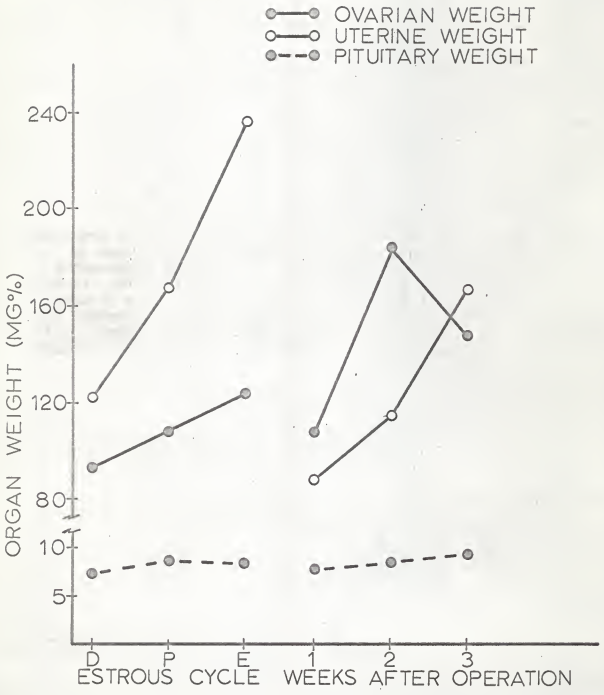
OVARIAN WEIGHT
UTERINE WEIGHT
PITUITARY WEIGHT
ORGAN WEIGHT (MG%)
240
200
160
120
80
10
5
D
P
E
1
2
3
ESTROUS CYCLE
WEEKS AFTER OPERATION

Fig. 14. Plasma and pituitary levels of luteinizing hormone (milliunits/mg or ml) during 3 phases of the estrous cycle and during 3 weeks after basolateral amygdaloid lesions. D = diestrus, P = proestrus, and E = estrus period in the estrous cycle. Each point represents the mean value of 4 (pituitary) of 2 assay animals (plasma).

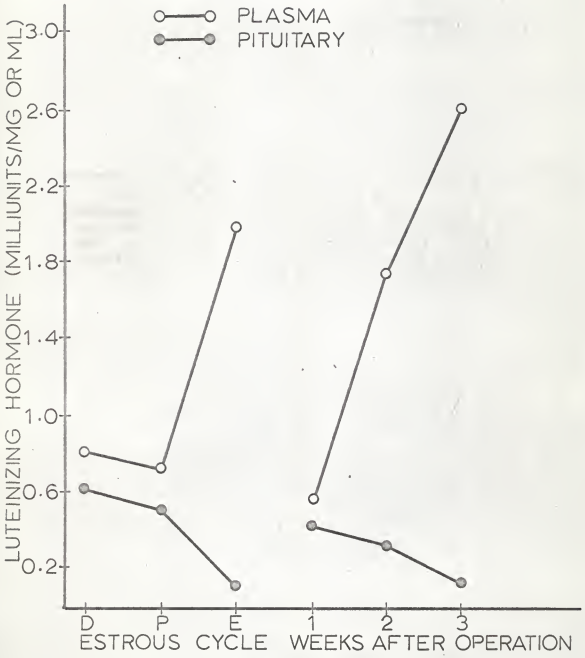
PLASMA
PITUITARY
LUTEINIZING HORMONE (MILLIUNITS/MG OR ML)
3.0
2.6
2.2
1.8
1.4
1.0
0.6
0.2
D
P
E
1
2
3
ESTROUS CYCLE
WEEKS AFTER OPERATION

Fig. 15. Effect of basolateral amygdaloid lesions on pituitary follicle stimulating hormone content. Stage of estrous cycle and weeks after lesioning are plotted on the abscissa. D = diestrus, P = proestrus and E = estrus stages in the estrous cycle. Each point represents the mean of 5 assay animals.

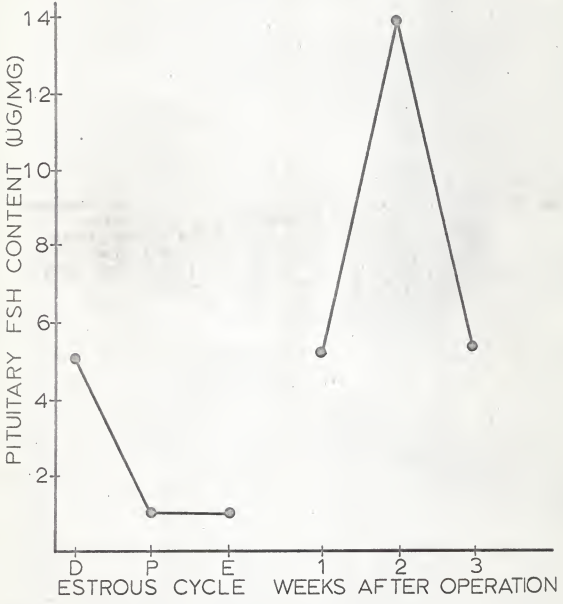
PITUITARY FSH CONTENT (µG/MG)
14
12
10
8
6
4
2
D
P
E
1
2
3
ESTROUS CYCLE
WEEKS AFTER OPERATION

Fig. 16. Standard dose response curve for follicle stimulating hormone* (FSH) calculated from the following equation: $Y = 605.322 + 274.108(\log X)$, where Y is uterine weight in mg/100 g of body weight and X is ng of FSH. Each point represents the mean response of 5 receptor animals.

* Obtained from the Endocrinology Study Section, National Institutes of Health.

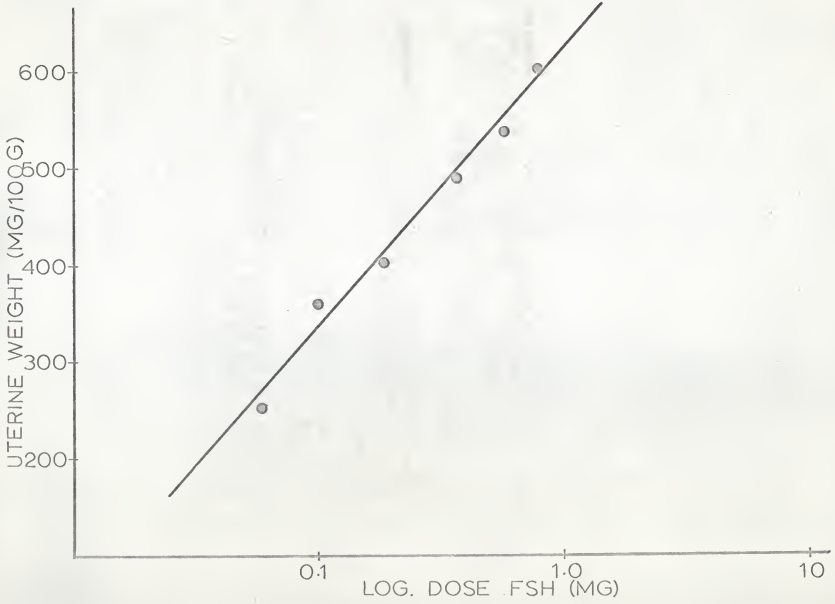
600
500
400
300
200
UTERINE WEIGHT (MG/100G)
0·1
1·0
10
LOG. DOSE FSH (MG)

Fig. 17. Standard dose response curve for luteinizing hormone* (LH) calculated from the following equation: Y = 67.656 - 27.552(log X), where Y is ovarian ascorbic acid in mg/100 g of tissue and X is µg of LH. Each point represents the mean response of 5 receptor animals.

* Obtained from the Endocrinology Study Section, National Institutes of Health.

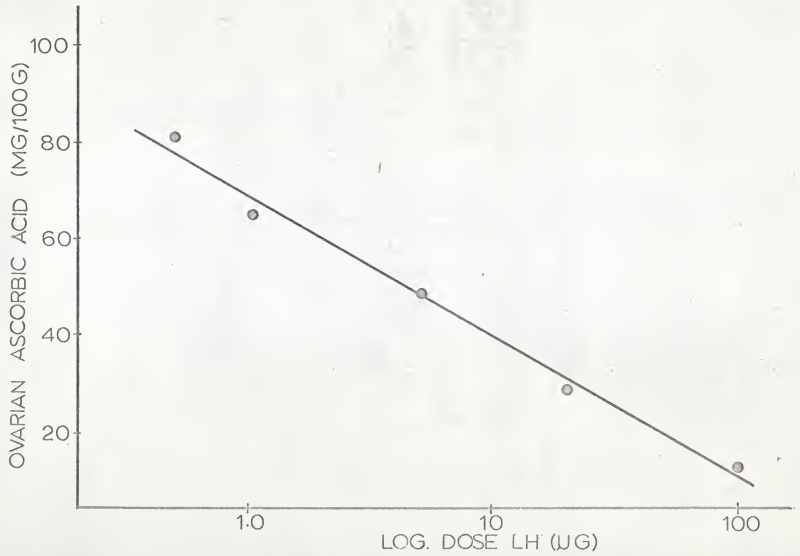
OVARIAN ASCORBIC ACID (MG/100G)
100
80
60
40
20
1·0
10
100
LOG. DOSE LH (μG)

Fig. 18. Ovarian section of a female deermouse taken two weeks after lesioning the basolateral amygdaloid nuclei, stained with Hematoxylin and Eosin. Note large corpora lutea.

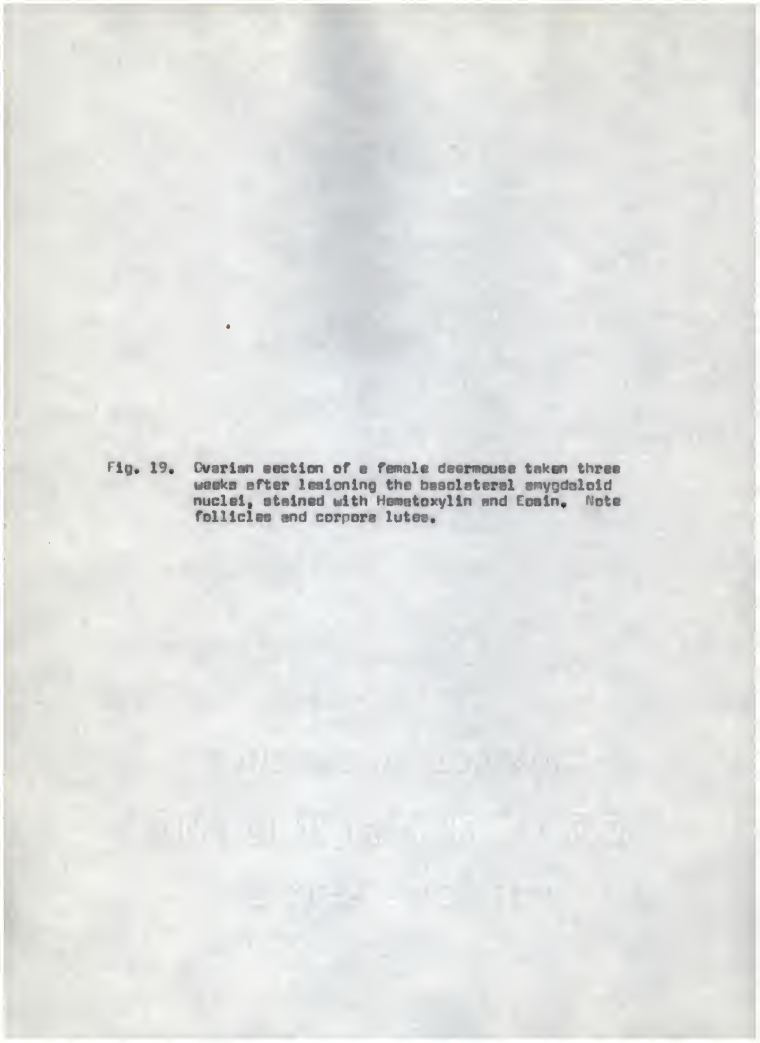

Fig. 19. Ovarian section of a female deermouse taken three weeks after lesioning the basolateral amygdaloid nuclei, stained with Hematoxylin and Eosin. Note follicles and corpora lutea.

DISCUSSION AND CONCLUSIONS

CONSTRUCTION OF THE STEREOTAXIC ATLAS

The forebrain of the deermouse (P. m. bairdii) was found to be anatomically similar in all respects to the rat (de Groot, 1959 and Zeman and Innes, 1963). Hypothalamic and thalamic nuclei of the deermouse occupied approximately the same position and were about equal in size in comparison to the brain of the rat. However, the amygdala of the deermouse appears to be larger in proportion to its brain in comparison to the rat, whereas the rat appears to have a greater cortex to brain ratio. Pribram (1966) contends that the amygdala functions as a hemostatic and orienting mechanism for the basic emotions of fighting, fleeing, feeding and sex. Therefore, it is not suprising that any structure that would function in making the animal better oriented to its enviroment should be larger than in an animal less dependent on its senses and environment. Since the deermouse is the progenitor of the rat, the rat is considered evolutionally a higher animal, and therefore, has a better degree of neural integration. Thus, the rat is expected to have a greater cortex to brain ratio.

EFFECT OF AMYGDALOID LESIONS ON ESTROUS CYCLING AND MATING BEHAVIOR

Experiments involving the effects of subtotal or total amygdalectomy on mating behavior are well documented. Both hyposexuality and hypersexuality, with or without impairment of other forms of behavior have been reported by Kling and Schwartz (1961), Goddard (1964) and Schreiner and Kling (1953). Bilateral or unilateral lesions confined to the medial and centromedial amygdaloid nuclei resulted in

a decrease in sexual (mating) behavior which agrees well with previous reports of the above investigators. While Schreiner and Kling reported a decrease in sexual behavior lasting as long as two weeks, this effect lasted three weeks in this experiment (termination of experiment).

Bilateral or unilateral lesions confined to the basolateral amygdaloid nuclei did not appear to appreciably alter mating behavior. Although only sixty-two percent of the animals with basolateral lesions mated during their first estrous cycle; this transient effect in itself, does not represent an alteration in normal mating behavior. It would not seem unreasonable to assume that the lag in mating was a result of general apathy due to general trauma (Wood, 1958).

Wood (1958) investigated the effect of discrete bilateral lesions in the lateral amygdaloid nuclei of cats and discovered that hypersexuality began only after a period of one to three weeks. Since the lateral nucleus is part of the basolateral complex (Kolegami et al., 1955), the effect of basolateral lesions should support Wood's experiment to a certain degree. Indeed this was the case, five animals that mated in diestrus mated two weeks after the operation. In addition, all five of these animals were not only lesioned in the basolateral nuclei but four of them had some portion of the lateral nuclei destroyed. Therefore, it appears that lesions confined to the posterior portion of the basolateral nuclei are relatively ineffective in producing hypersexuality in this species. Furthermore, at least some lateral nuclear destruction is necessary to produce a hypersexual response.

Since hippocampal damage was observed in animals with both types of lesions, it was concluded that the integrity of this structure exerts little if any direct control on mating behavior in this species.

Anand et al. (1959), Pribram and Bagshaw (1953), Schreiner and Kling (1953) and Terzian and Ore (1955) observed hypersexuality in bilateral amygdalectomized animals, whereas, Green and his co-workers (1957) and Kling and Schreiner (1961) reported that amygdalectomy had no effect on sexual behavior. The present investigation supports the view that the medial amygdaloid nuclei exert an important influence in maintaining normal mating behavior while the basolateral amygdaloid complex acts on an inhibiting center for controlling the mating urge. In addition, these data suggest that the mating drive originates in the medial amygdaloid nuclei but is inhibited by the nuclei of the basolateral complex in a reciprocal manner. Therefore, the upsetting of this balance may possibly explain the varied effects in behavior attributed to dysfunction of the amygdala.

EFFECT OF BASOLATERAL AMYGDALOID LESIONS ON PITUITARY AND PLASMA GONADOTROPINS

Pituitary gonadotropin concentrations were found to be consistant with previous findings in the rat (Schwartz and Calderelli, 1965; Meric et al., 1965 and Corbin, 1966) and pig (Melampy et al., 1966 and Anderson et al., 1966). In addition, Melampy and his co-workers and Anderson and his co-workers employing similar assay procedures reported comparable pituitary FSH:LH ratios in the pig. When plasma LH is corrected to ug per animal the values agree well with plasma values reported by Schwartz and Calderelli (1965) for the rat. Since

no previous work appears in the literature on plasma LH concentrations after amygdaloid lesions, it is difficult to compare the effect of this treatment on plasma LH levels.

Bilateral lesions confined to the basolateral amygdaloid nuclei yielded an increase in plasma LH from one to three weeks following lesions with an accompanying decrease in pituitary LH (Fig. 14). The changes recorded in organ weights in animals with lesions can best be correlated with altered secretion rates of pituitary and ovarian hormones. Uterine tissue development is primarily dependent on estrogen secretion from FSH stimulated ovarian follicles, while increases in ovarian weight is primarily attributed to formation of corpora lutea under the influence of LH and possibly Luteotropic hormone (LTH) in this animal (Taleisnik and McCann, 1961). Therefore, since the uterine tissue failed to gain weight in proportion to normal animals during the estrous cycle, it can be concluded that a sufficient amount of FSH was absent from the systemic circulation or that estrogen synthesis was altered in the ovary of lesioned animals. The former assumption appears to be more consistant with the present data in view of the fact that during one to two weeks following lesions pituitary FSH content increased indicating little if any release (Fig. 15). The rise in ovarian weight in the lesioned animals can be correlated with formation of corpora lutea (Fig. 18) as evidenced by a sharp increase in plasma LH and probably LTH. However, between two and three weeks following lesions, the ovarian weight decreased while the uterine weight increased to almost proestrus levels. The drop in ovarian weight can best be interpreted as resulting from increased secretion of FSH (evidenced by

a drop in pituitary FSH content) which would permit partial resorbtion of corpora lutea with increased production of follicles and, therefore, estrogen (Fig. 19). With the presence of sufficient quantities of estrogen in the systemic circulation, the uterine tissue becomes properly stimulated to develop. The sharp rise in uterine weight (Fig. 13) from two to three weeks adds support to the above statement. However, since plasma FSH was unable to be determined, due to limited amount of plasma, the only evidence in support of these statements is the biphasic response in pituitary FSH in the treated animals.

Another hypothesis (but less convincing) can also be described in order to explain the train of events associated with basolateral amygdaloid lesions. Martin et al. (1958) discovered that in amygdalectomized cats and dogs, plasma levels of adrenal corticoids dramatically increased and sometimes a new type of corticoid was produced. If a qualitative change in estrogen occured in the lesioned animals, the uterine tissue may not have been able to metabolize the new estrogen and, therefore, not develop. The lack of normal estrogen then would trigger the pituitary to produce FSH through the negative feedback mechanism in the hypothalamus (Flerko, 1963), thus accounting for the increase in pituitary FSH. Exactly what effect this qualitative shift in estrogen would have on pituitary synthesis and release of LH cannot be elucidated with the present data.

The possibility that LTH is secreted as a result of basolateral amygdaloid lesions would certainly explain the formation and persistence of corpora lutea, but this hypothesis seems unlikely since the

uterine tissue failed to increase in weight. If LTH were secreted the uterine tissue should have been comparable in weight to animals in pseudopregnancy.

Previous investigators have demonstrated that ovulation (LH release) can be induced by stimulation, lesioning or ablation of the amygdala or as a result of implantation of acid extracts of amygdalae into the tuber cinerium. On Hillard's assumption that LH induces steroidogenesis in the ovary (Hillard et al., 1964), Endroczi and Hillard (1965) implanted acid extracts of rabbit or dog amygdalae into rabbit median eminence and stimulated progesterone secretion from the ovary. By stimulating the medial or centromedial amygdaloid nuclei Shealy and Peele (1957) and Koikegami et al. (1954) induced ovulation in rabbits thus implicating the amygdala in the regulation of LH secretion. Bunn and Everett (1957) also produced the same effect but did not indicate what region in the amygdala was necessary for the elaboration of ovulation. This investigator does not feel that the above data contradict the present experiment involving lesions, since the above investigators stimulated an area not involved in this experiment. Since a rich network of nervous connections is known to exist between amygdaloid nuclei (Gloor, 1964), it is not suprising that dysfunction of one nucleus may effect another. Thus, stimulation of one nuclei can activate another which then produces the observed effect.

Shealy and Peele (1957) lesioned the basolateral and lateral amygdaloid nuclei in rabbits and induced ovulation which is in good agreement with the present findings. Since ovulation was not used as a parameter in this experiment the amount of LH released from the

pituitary cannot be compared. However, since a tonic discharge of LH is needed to induce ovulation (Flerko, 1963) and corpora lutea were found in the lesioned animals, it seems reasonable to assume that greater than normal or normal amounts of LH were secreted from the pituitaries of the basolateral lesioned animals. As to how sustained was the LH release and at what rate was LH released cannot be determined by this experiment or previous experiments.

The release of pituitary LH as a result of amygdaloid lesions should not be attributed to general trauma. Although Talaisnik et al. (1962) noted a significant depletion of ovarian ascorbic acid from animals one hour following puncture of the neo-cortex with an electrode, and thus attributed the release of LH to actual spreading of a stimulus from the site of injury to the hypothalamus, Bunn and Everett (1957) maintained that gross asymetric lesions not in the amygdala do not induce ovulation. It would seem that the mechanism by which an impulse induces LH release in the neo-cortex is different than the mechanism that operates in the amygdala for LH release. Since the amygdala is relatively close to the hypothalamus, the possibility of trauma causing LH release cannot be overlooked. However, it is unlikely that a traumatic stimulus would be sustained for three weeks as these data indicate. In addition, animals that were lesioned in the posterior portion of the basolateral or medial amygdaloid nuclei (experiment one, page 16) cycled normally, thus supporting Bunn and Everett's assumption. Had LH been released constantly from the pituitary, normal cycling would have ceased and the animals would have gone into constant diestrus as in the case of the lesioned animals in this experiment.

In describing a specific mechanism of action for amygdaloid regulation of pituitary gonadotropins, the route of propagation for a stimulus or lack of stimulus, in the case of lesions, should be traced from its origin in the amygdala to the pituitary. It is uncertain whether the amygdala exerts its effect directly on the pituitary, hypothalamus or indirectly on the hypothalamus via another subcortical or even cortical structure. In addition, it is not known whether the impulse originating in the amygdala is neural, neurosecretory or hormonal in nature. Pribram (1963) and Gloor (1964) described many neural pathways by which an impulse originating in the amygdala or from the amygdala can travel directly to various gonadotropin regulating centers in the hypothalamus or indirectly to the hypothalamus via other subcortical structures. Employing selective stains for neurosecretory granules and serotonin assays, Moore and his coworkers (1965) have demonstrated several possible pathways by which neurosecretory substances can reach the hypothalamus from the amygdala. Evidence for hormonal (steroidal) stimulation of the hypothalamus via the amygdala seems to be ruled out as a possible pathway by Michael's failure to alter sexual behavior in cats with estrogen implants in the amygdala (1965). A pathway by which gonadotropins are regulated by the amygdala will not be suggested here except to state that evidence for both neural and neurosecretory pathways exist from the amygdala to various other components of the limbic system.

Various lesion experiments in the median eminence and anterior hypothalamic area implicate the hypothalamus as the possible target area of amygdaloid activity in gonadotropin regulation. The particular

syndrome associated with basolateral amygdaloid lesions has been demonstrated by Taleisnik and McCann (1961) in animals with median eminence lesions. Constant diestrus, corpora lutea formation, decrease in uterine and ovarian weight and low pituitary LH levels were associated with hypothalamic lesions in female rats. However, no detectable plasma LH was found in these animals which the authors attributed to impaired LH synthesis. They also concluded the lower uterine weight was due to low levels of FSH and probably LH. However, it should be pointed out that LH was neither detected in normal plasma nor in the plasma of lesioned animals. Therefore, an accurate conclusion about relative plasma LH levels was not drawn. Since the rise in pituitary FSH in lesioned animals may be an effect of progesterone inhibition (negative feedback) caused by high plasma titers of LH and, therefore, not a result of lesions, its hypothalamic regulating center will not be defined here until plasma FSH levels are determined. The low uterine weight observed in lesioned animals from one to two weeks suggest that this gonadotropin is decreased or absent from circulation, which agrees with the results of Taleisnik and McCann's median eminence lesioned rats.

In summing up the above data, it appears that the function of the basolateral amygdaloid nuclei is to inhibit the release of pituitary LH until it is needed (i.e. for ovulation) via the hypothalamus. Thereby, removal of these nuclei results in a continued release of LH by the pituitary which leads to the particular syndrome described in this thesis. The actual synthesis of LH does not seem to be impaired. Whether this effect is a specific result of the lesions or a result of disorganization of other behavioral patterns cannot be determined until

further information is obtained from stimulation experiments involving the limbic system, neurosecretory studies in the amygdala and other parts of the brain and other behavioral and endocrine related experiments for this species.

SUMMARY

Bilateral or unilateral electrolytic lesions, with or without hippocampal damage, confined to the posterior portion of the medial amygdaloid nuclei in the female deermouse (P. m. bairdii) resulted in normal estrous cycling but no mating, whereas lesions confined to the posterior portion of the basolateral amygdaloid nuclei resulted in both normal estrous cycling and mating. In addition, five animals with basolateral and lateral amygdaloid lesions mated in diestrus, which was interpreted as a hypersexual response as a result of the treatment. These data demonstrate that the integrity of both the medial amygdaloid nuclei and the nuclei of the basolateral amygdaloid complex are necessary in normal regulation of sexual behavior in this species. This author suggests that the mating drive originates in the medial amygdaloid nuclei while being inhibited by the nuclei of the basolateral complex in a reciprocal manner. Therefore, the removal of the basolateral and lateral nuclei allows for an increased expression of the functions originating in or mediated through the medial amygdaloid nuclei. The wide range of behavioral phenomena attributed to dysfunction of the amygdaloid complex can then be explained by upsetting this inhibitory-facilatory balance.

Bilateral electrolytic lesions confined to the anterior portion of the basolateral amygdaloid nuclei resulted in an increase in plasma luteinizing hormone (LH) throughout the observed postoperative period with an accompanying decrease in pituitary LH. Synthesis of LH did not seem to be impaired while release was enhanced. The formation of corpora lutea in the ovary were the result of increased circulatory levels of LH while the persistance of corpora lutea over the three week postoperative period was probably due to the secretion of both LH and luteotropic hormone (LTH). Thus, the observed increase in plasma LH correlates well with the observed increase in ovarian weight during the postoperative period.

Pituitary follicle stimulating hormone (FSH) content was found to increase from one to two weeks after the operation indicating little if any release. However, increases in uterine weight and histological changes in the ovaries of lesioned animals demonstrate release of pituitary FSH from two to three weeks after the operation. The inhibition of FSH secretion from one to two weeks following the lesions could not be explained by the above data alone. Further evidence would be necessary to establish whether the lag in FSH secretion was due to the lesions or simply to the negative feedback effect of progesterone due to either LH or LTH stimulation of corpora lutea.

The nature of the mechanism by which the amygdala exerts its influence on pituitary gonadotropins is discussed. These and other data suggest the basolateral amygdaloid complex functions as an inhibitor for the release of pituitary LH via the hypothalamus either directly through established neural and neurosecretory pathways or

indirectly through other subcortical structures. Thereby, removal or destruction of this inhibitory mechanism results in a particular syndrome described in this thesis. Whether this effect is a specific result of lesions or a result of disorganization of other behavioral patterns cannot be determined until further information is obtained from stimulation experiments involving other structures in the limbic system, neurosecretory studies in the amygdala and other parts of the brain and other behavioral and endocrine related experiments for this species.

ACKNOWLEDGMENTS

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LITERATURE CITED

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ADDENDUM

Klinefelter, H. F., Albright, F. & Griswold, G. C. (1943). Experience with quantitative test for normal or decreased amounts of follicle stimulating hormone in urine in endocrinological diagnosis. J. Clin. Endocrinol. 3:529-544.

EFFECT OF AMYGDALOID LESIONS ON ESTROUS BEHAVIOR AND GONADOTROPIN SECRETION IN PEROMYSCUS MANICULATUS BAIRDII

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The effect of electrolytic lesions in the amygdaloid complex of the female deermouse (Peromyscus maniculatus bairdii) was investigated to further elucidate the role of the amygdala in the regulation of mating behavior and gonadotropin secretion.

Accurate placement of lesions demanded that a stereotaxic atlas of the forebrain be constructed for this species. The stereotaxic atlas consisted of twenty transverse diagrams and two longitudinal parasagittal diagrams illustrating various representative brain structures. The internal anatomy of the brain of P. m. bairdii was found to be similar to the neuroanatomical structures described for the rat.

Bilateral or unilateral lesions, with or without hippocampal damage were confined to the posterior portion of the medial or basolateral amygdaloid nuclei. Lesions in the medial amygdaloid nuclei inhibited normal mating without impairment to normal estrous cycling, whereas, thirty-three percent of the animals lesioned in the basolateral nuclei exhibited a lag of one estrous cycle before mating, compared to normal animals. The latter lesioned animals also were shown to cycle normally. Five animals lesioned in the basolateral and lateral amygdaloid complex mated in diestrus, which was interpreted as a hypersexual response as a result of the treatment. The present investigation supports the assumption that the integrity of both the medial and nuclei of the basolateral amygdaloid complex are essential in maintaining normal mating behavior in this species. In addition, these data suggest the mating drive originates in the medial amygdaloid nuclei while being inhibited by the nuclei of the basolateral amygdaloid complex in a reciprocal manner. Therefore, the wide range of behavioral phenomena

attributed to dysfunction of the amygdala can possibly be explained by upsetting this inhibitory-facilatory balance.

Bilateral lesions confined to the anterior portion of the basolateral amygdaloid nuclei yielded an increase in plasma luteinizing hormone (LH) from one to three weeks following the operation with an accompanying decrease in pituitary LH. During the postoperative three week period, pituitary follicle stimulating hormone (FSH) content increased 240% at two weeks from a normal diestrus level at one week, then, decreased to a comparable diestrus value at three weeks following the operation. Changes in ovarian and uterine weight were found to be indicative of fluctuating levels of pituitary and plasma gonadotropins. The dramatic rise in ovarian weight two weeks following the operation was attributed to luteinization of existing ovarian follicles due primarily to increased circulatory levels of LH and possibly luteotropic hormone (LTH).

The nature of the mechanism by which the amygdala exerts its influence on pituitary gonadotropins is discussed. These and other data suggest the basolateral amygdaloid complex functions as an inhibitor for the release of pituitary LH via the hypothalamus either directly through established neural and neurosecretory pathways or indirectly through other subcortical structures. Thereby, removal or destruction of this inhibitory mechanism(s) (i.e. lesions or ablation) results in a particular syndrome described in this thesis. Whether this effect is a specific result of lesions or a result of disorganization of other behavioral patterns cannot be determined until further information is obtained from stimulation experiments involving the

limbic system, neurosecretory studies in the amygdala and other parts of the brain and other behavioral and endocrine related experiments for this species.