EFFECT OF BASOLATERAL AMYGDALOID LESIONS ON PROLACTIN SECRETION IN *PEROMYSCUS MANICULATUS BAIRDII*

by

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INTRODUCTION

Hormonal control of the development and maturity of the mammary gland has been investigated for nearly eighty years. Very little progress was made in elucidation of this control until two workers at Bouin's laboratory in Strasbourg, France, induced milk secretion in ovariectomized virgin rabbits with pituitary extract (Strickler and Grueter, 1928; 1929). The lactogenic extract was named prolactin and a bio-assay was developed utilizing the increase in weight of the pigeon crop-sac as the end point (Riddle, Bates and Dykshorn, 1933). To explain this lactogenic action of anterior pituitary extract, Turner and co-workers developed the "mammogen theory", which proposed two hypothetical pituitary mammogens (Mixner, Lewis and Turner, 1940). This theory which implicated two pituitary factors for mammary gland development was partially correct, but their alternate hypothesis which implicated synergism between estrogenic steroids and pituitary factors as a prerequisite for complete lactogenic activity was more correct. Eventually, it was shown that for complete lobulo-alveolar development of the mammary gland in the hypophysectomized rat, estrogen, progesterone, adrenal corticoids, prolactin and growth hormone were required (Lyons, Li, Cole and Johnson, 1953; Chen, Johnson, Lyons, Li and Cole, 1953).

The original bio-assay for prolactin (Riddle et al., 1933) is the most widely used of all assays for lactogenic material.

The sensitivity of this assay can be increased by using adult rather than juvenile pigeons with a seven-day rather than a four-day injection schedule (Bates, Garrison and Cornfield, 1963). Numerous other assays for prolactin can be found in the literature, but they are either very laborious or they lack sensitivity. Some of the more prominent are: P^{32} uptake by the stimulated crop-sac (Damm, Pipes, von Berswordt-Wallrabe and Turner, 1961); measurement of the diameter of the crop response (Grosvenor and Turner, 1958); dried weight of the pigeon crop mucosa (Kanematsu and Sawyer, 1958); response of rabbit intraductal tissue (Lyons, 1937; Chadwick, 1963); prolongation of diestrus in mature virgin mice (Kovacic, 1962); response of the corpora lutea cells of hypophysectomized rats treated with human chorionic gonadotropin and pregnant mare's serum (Wolthius, 1963); increase in β-glucuronidase activity in rat and mouse testes (Evans, 1962), and a radioimmunological assay based on I^{125}-labelled mouse pituitary tumor protein (Kwa and Verhofstad, 1966). A sensitive blood assay for prolactin has yet to be developed.

During the last decade, there has accumulated a body of literature supporting the theory of hypothalamic control of prolactin synthesis and release (Desclin, 1949, 1950, 1956; Everett, 1954, 1956; Nikitovitch-Winer and Everett, 1958; Haun and Sawyer, 1960; Nikitovitch-Winer, 1965). There is a substance, prolactin inhibiting factor (PIF), which is released from the hypothalamus and appears to be an entity different

from the releasing factors of other pituitary gonadotropins (Schally, Meites, Bowers and Ratner, 1965). This PIF originates from a different area of the hypothalamus than does LHRF or FSHRF (Rothchild, 1960; Corbin, 1966). Both the median eminence and posterior-tuberal regions of the hypothalamus exert a profound influence on prolactin synthesis and release (Kanematsu and Sawyer, 1963; Kanematsu, Hillard and Sawyer, 1963). However, it is difficult to pinpoint the origin of PIF because the control of other hormones necessary for lactogenic action is also hypothalamic (Meites, Nicoll and Talwalker, 1963; Corbin and Cohen, 1966). The posterior median eminence region of the hypothalamus seems to be the most likely locale for production of PIF. Estradiol implants in this region result in increased synthesis and storage of prolactin while estradiol implants in the adenohypophysis cause a direct action for the release of prolactin. This situation is similar to that occuring in late gestation when placental production of estrogens is high (Lyons, 1958).

Although the amygdala is anatomically part of the limbic system, it has afferent connections with subcortical structures such as the hypothalamus (Goddard, 1964; Crosby, Humphrey and Laurer, 1962). In the human, this connection is via two fiber systems: (1) the stria terminalis and (2) the amygdalo-hypothalamic and amygdaloseptal tract (Klinger and Gloor, 1960). The amygdala has five discreet areas or nuclei; medial, cortical, central, lateral and basolateral. The nuclei are divided into

two groups with the phylogenetically older group, central cortical and medial nuclei, influencing various autonomic responses (sniffing, chewing, gastrointestinal responses, and some respiratory and blood pressure alterations). The lateral and basolateral nuclei compose the younger phylogenetic group and are involved in behavorial phenomena such as attention, fear and anger (Ursin and Kaada, 1960). The basolateral group, via the hippocampus and fornix, discharges into the mammillary region of the hypothalamus. This amygdaloid group also has connections to the preoptic and ventromedial hypothalamic areas via the stria terminalis (Crosby et al., 1962). These afferent connections afford the amygdala considerable means by which hypothalamic function can be regulated.

There is neuroanatomical and neurophysiological evidence to indicate that the amygdala integrates all the major senses. Thus, the amygdala exerts an influence on several autonomic and somatic phenomena (Goddard, 1964). Among these are: movement of the face and jaws (Frost, Baldwin and Wood, 1958); respiratory inhibition and blood pressure depression (Wood, Schottelius, Frost and Baldwin, 1958); Salivation pupillodilation, micturition, defecation and piloerection (Ursin and Kaada, 1960); feeding and grooming (Fonberg and Delgado, 1961; Schwartz and Kling, 1964); fear, anger and arousal (Fonberg, 1963; Ursin, 1960) and sexual behavior (Goddard, 1964; Eleftheriou and Zolovick, 1966). Although the amygdala does not control the various sub-cortical areas from which these somato-autonomic

responses are derived, it acts as a modulator on processes dependent upon these sub-cortical structures (Gloor, 1955). Bilateral ablation of the amygdala, in most cases results in hypersexuality, especially in the male (Goddard, 1964). This increased sexual behavior apparently is non-specific since lesioned males will attempt to copulate with non-estrus females, males and even inanimate objects. Ablation of the medial and lateral groups of the amygdala has been shown to increase especially sexual behavior (Wood, 1958; Eleftheriou and Zolovick, 1966). There is a period during the first week after the operation when sexual activity decreases; however, this can be attributed to the lethargic state of the animal which is generally observed following amygdaloid lesions (Anand and Brobeck, 1952; Schreiner and Kling, 1953; Koikegami et al., 1955; Yamada and Greer, 1960). Hypersexuality in these animals is confusing as amygdaloid ablation in the male results in a significant atrophy of the testes (Yamada and Greer, 1960; Kling, Orbach, Schwartz and Towne, 1960), while in the female where sexual activity is seldom altered, the ovaries remain either unaffected or show an increase in weight (Yamada and Greer, 1960; Kling, 1965; Zolovick, Norman and Eleftheriou, 1967). The most apparent consequences of amygdaloid lesions in the female animal is the disruption of maternal behavior. The infant monkey has little chance of survival if the mother has been amygdalectomized (Walker, Thompson and McQueen, 1953; Masserman, Levitt, McAvoy, Kling and Pechtel, 1958).

Discreet lesions placed in lateral amygdaloid nuclei result in non-cyclic behavior of the female rat (Flerko and Bardos, 1966) and deermouse (Eleftheriou and Zolovick, 1966). Presumably, this prolongation of diestrus results from the altered gonadotropic (FSH and LH) levels which are present in animals with lesions in the lateral group (Eleftheriou, 1967; Eleftheriou, Zolovick and Norman, 1967; Zolovick et al., 1967). Both FSH and LH content of the pituitary and plasma was shown to increase with basolateral amygdaloid lesions. This supports the theory that amygdaloid influence on hypothalamic control of gonadotropin secretion is one of inhibition. However, this theory excludes amygdaloid influence on hypothalamic control of prolactin secretion. The hypothalamus depresses the production of prolactin from the adenohypophysis (Desclin, 1949, 1950; Everett, 1954, 1956), which is converse to the control of FSH and LH (Corbin, 1966; Corbin and Cohen, 1966). The role of the amygdala in regulating hypothalamic control of prolactin secretion is not known, probably because of the lack of an assay sufficiently sensitive to measure physiological levels of prolactin.

Flerko and Bardos (1966) propose a stimulatory influence for the amygdala on the hypothalamic inhibition of prolactin secretion. If partial destruction of the amygdaloid system could decrease the hypothalamic production of PIF, prolactin secretion would be enhanced. This increased production and release of prolactin would maintain newly formed corpora lutea

which secrete progesterone sufficient to induce and maintain "pseudopregnancy-like" prolongation of diestrus.

This report deals with lesions in the basolateral amygdaloid nuclear group and their effect on prolactin levels in the pituitary and plasma of the deermouse.

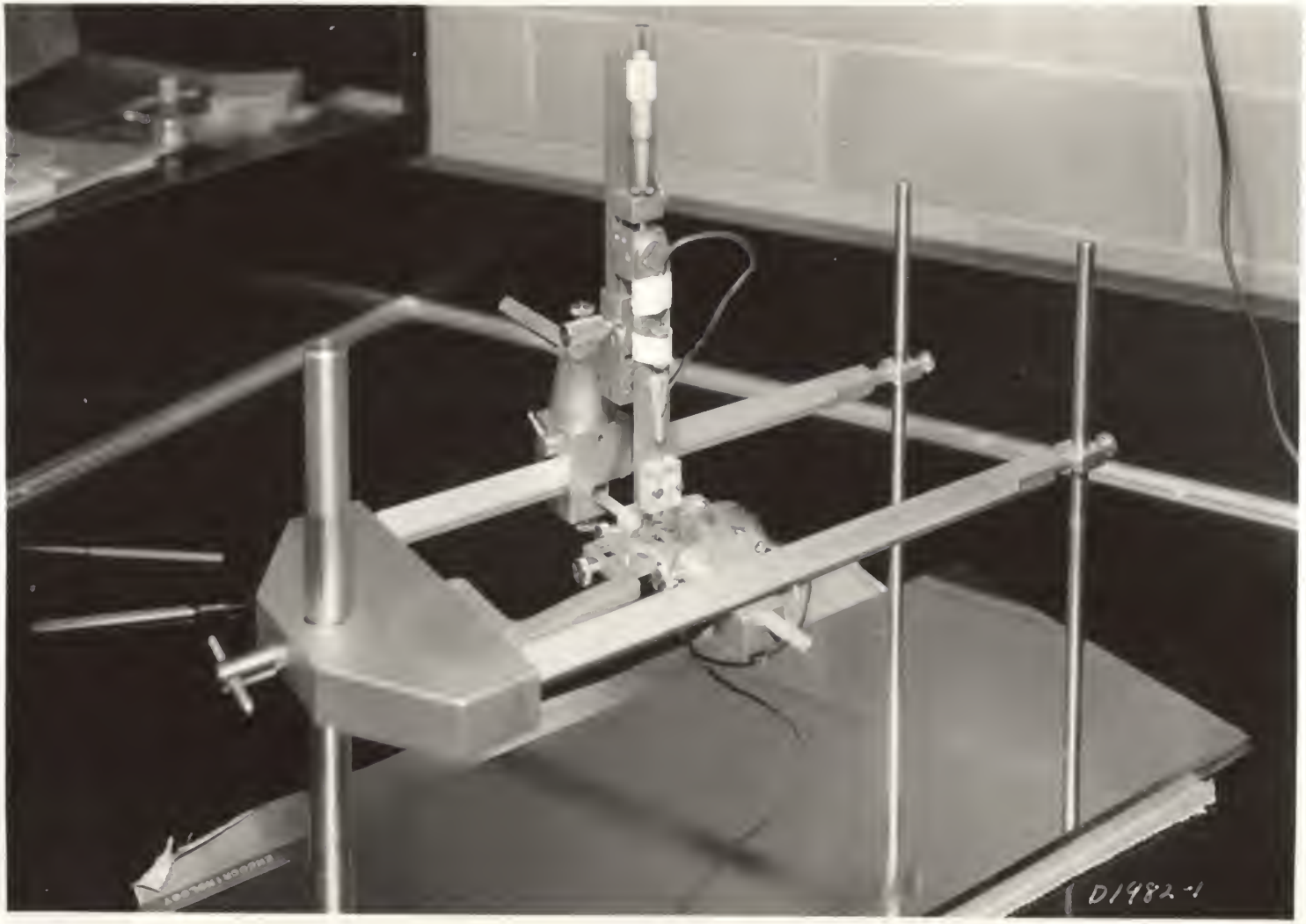
MATERIALS AND METHODS

LESIONS

Adult female deermice (Peromyscus maniculatus bairdii) weighing 13-17 grams and 70-90 days old were anesthetized with 1.2 mg sodium pentobarbitone administered intraperitoneally. The deermice then were oriented in a rat stereotaxic apparatus (Trent Wells Jr.) so that the electrode was centered at the most posterior portion of the junction of the lambdoidal and sagittal sutures (Fig. 1). From this point, the electrode was moved to the area of the desired coordinates (forward, 5.2 mm; lateral, 2.7 mm; vertical, 5.5 mm) for the basolateral amygdaloid nucleus (Eleftheriou and Zolovick, 1965). A LM-3 Radio Frequency Lesion maker (Grass Instruments, Inc.), discharging 20 uA of current for 30 seconds was used to produce lesions by electrocoagulation. The electrodes were insulated with three layers of varnish and oven dried at 190° C (Whitfield, 1964). A 0.5 mm portion of varnish was scraped from the tip of the electrode and this exposed portion placed at the site of the desired lesion discharged the necessary current to produce the lesion through electrocoagulation.

Post-operatively, the mice were housed three per cage in clean plastic cages, and maintained on water and standard rat laboratory chow ad libitum. Vaginal smears were taken daily, and those mice with normal or near-normal cycles were not used because non-cyclic behavior has been shown to be charac-

Fig. 1. Lateral view of P. m. bairdii in position in a modified rat stereotaxic apparatus.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teristic of animals with lesions in this area (Eleftheriou and Zolovick, 1966; Flerko and Bardos, 1966).

COLLECTION OF TISSUE

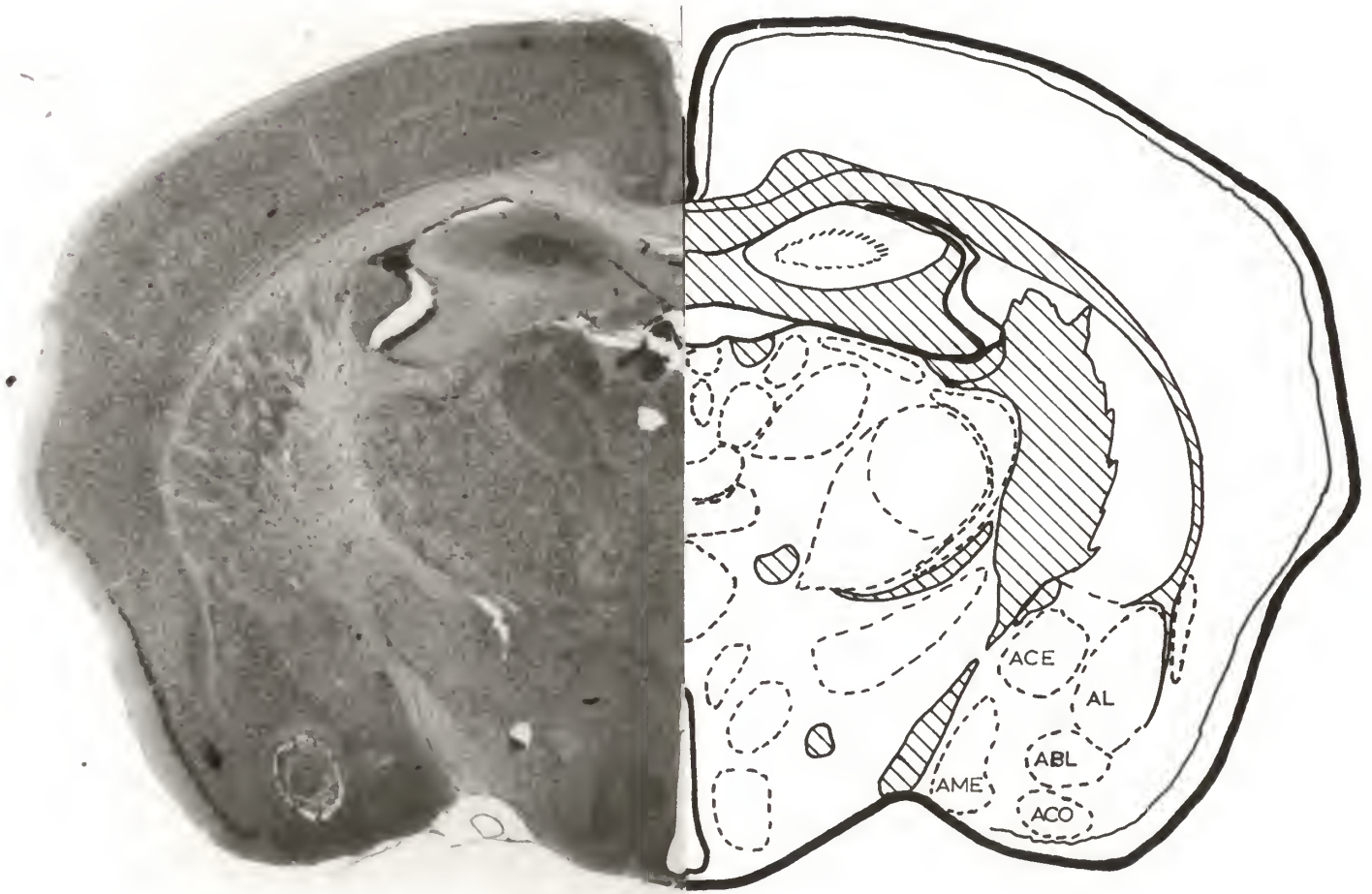
Terminally, the non-cyclic mice were divided into three groups per period, with thirty mice per group. Tissues were collected at one day, one, two and three weeks post-lesion, and during diestrus, proestrus and estrus stages of the estrous cycle. The orbital sinus was entered and blood was collected in one ml heparinized syringes. The blood was kept in an ice bath during collection. Plasma was obtained by centrifugation at 1000 x g for 15 minutes in a refrigerated centrifuge and was frozen for later analysis. Body weights were taken and the pituitaries quickly removed and placed in 0.5 ml of ice cold physiological saline. Pituitaries from each group of 30 mice were combined and stored in the freezer until analysis.

Ovaries were removed, trimmed of excess tissue and weighed to the nearest 0.1 mg on a Roller-Smith torsion balance. The uterine tubes also were removed, cleared of excess tissue, blotted and weighed. Brains were removed and examined macroscopically for placement of lesions. Sections of the brain were made for histological examination (Fig. 2).

ASSAY OF TISSUE

Plasma from each group was prepared just before use in three different concentrations. The highest concentration was undiluted plasma, the second concentration was one-half plasma and one-half saline and the third concentration was one-fourth

Fig. 2. Composite diagrammatic-photomicrographic transverse section illustrating representative structures and basolateral amygdaloid lesions in the brain of *P. m. bairdii*.

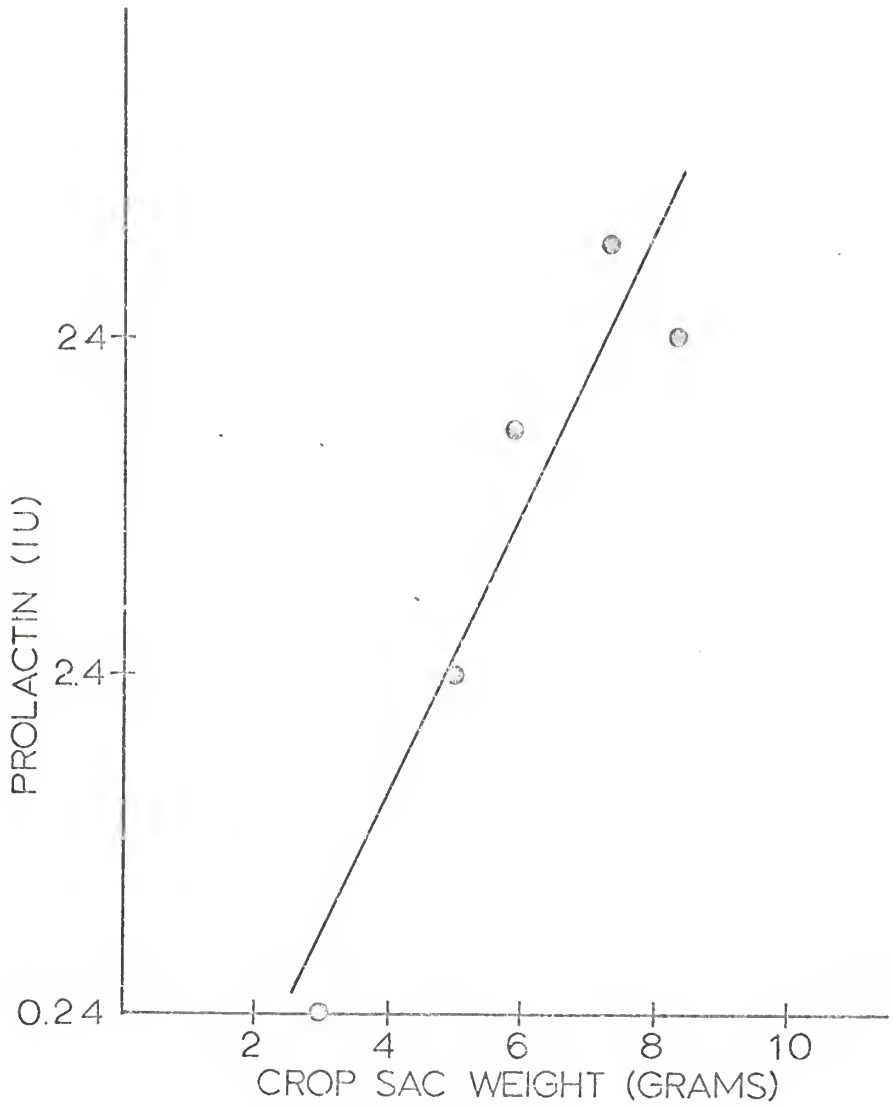

ACE
AL
ABL
AME
ACO

plasma and three-fourths saline. Pituitaries were homogenized in ice-cold saline and prepared in three concentrations: 1.0 mg tissue/0.4 ml saline, 0.5 mg tissue/0.4 ml saline and 0.25 mg tissue/0.4 ml saline.

Prolactin content of plasma and pituitary tissue was determined by a bio-assay using pigeon crop-sac weight increase as the end point (Riddle, Bates and Dykshorn, 1933). Adult pigeons were used to increase the sensitivity of the assay (Bates, Garrison and Cornfield, 1963). All 207 pigeons were retired breeders, five years and older, obtained from Palmetto Pigeon Plant, Sumter, South Carolina. During the experiment the pigeons were housed in metal isolation cages and maintained on a mixed grain and water diet. Each dilution of pituitary and plasma was injected at the same time each day for four consecutive days. Each injection was 0.4 ml of the assay material and was administered intramuscularly in the pectoral muscle adjacent to the crop-sac. On the fifth day, the pigeons were decapitated and the crop-sac removed. The contents of the crop-sac were removed and fat was trimmed before weights were taken. The weight of the crop-sac was determined to the nearest 0.01 gram. Control weights were determined using saline injections.

A standard curve was constructed using prolactin concentrations of 0.24 IU, 2.4 IU, 12.0 IU, 24.0 IU and 48.0 IU per bird with five birds per point (Fig. 3). Prolactin (NIH P-S-7) was obtained through the courtesy of the Endocrine section of the National Institutes of Health, Bethesda,

Fig. 3. Standard dose response curve for prolactin calculated from the collowing equation: $Y = 0.01 (X - 2.433/2.377)$, where Y = IU of prolactin and X = weight of crop-sac. Each point represents the mean response of 5 receptor animals.


PROLACTIN (IU)
24
2.4
0.24
2
4
6
8
10
CROP SAC WEIGHT (GRAMS)

Maryland. Prolactin concentrations per mg of tissue or per ml plasma was determined using the following formula derived from the standard curve: $\log Y = \frac{X - 2.433}{2.377} \times 0.01$ where Y = IU of prolactin and X was the experimental crop-sac weight. The intragroup values were then combined and an average value determined. The mean value in IU of prolactin was determined for each time period investigated and represented graphically.

Fixation of the brains for histological examination was performed according to a modification of the technique of McClung and Allen (1950). Anesthetized mice were perfused with saline solution followed by Telly-Fekete fixative. After perfusion, the mouse was wrapped in wet paper towels and placed head down for two to three hours. The brain then was removed and placed in the same fixative for twenty-four hours. The brain was dehydrated with dioxane, embedded in paraffin and sectioned at 15 μ. The sectioned tissue was stained with Cresyl Violet Blue by the method of Powers and Clark (1955).

RESULTS

Approximately ninety percent of the deermice lesioned bilaterally in the basolateral amygdaloid nucleus exhibited non-cyclic estrous behavior. Vaginal smears taken from lesioned animals were of the type obtained during pseudopregnancy.

The lesions were discrete, involving only the basolateral complex of the amygdala (Fig. 2). Extremely small lesions placed in this area did not result in the lethargic post-lesion reaction characteristic of bilateral destruction of amygdala. This lethargic status of animals lesioned in the amygdaloid complex was found to be correlated with injury to the cortical amygdaloid nucleus rather than the basolateral complex.

Effect of bilateral destruction of the basolateral amygdaloid nucleus on ovarian and uterine weights is shown in figure 4. Organ weights are expressed in mg tissue per 100 grams of body weight (mg%) to correct for variation in body weight.

Estrous cycle control weights of ovaries were 104 mg% at diestrus, increasing to 128 mg% at proestrus and 112 mg% at estrus. Values for experimental animals were generally lower than control values. One week following the operation, the ovarian weight was 88 mg%. At two weeks, the ovarian weight had increased to 110 mg% and at three weeks following the operation the ovarian weight was 105 mg%, essentially that of the diestrus value.

TABLE I

Values for pituitary and plasma prolactin concentrations and ovarian and uterine weights during the estrous cycle.

Estrous Cycle Stage	Prolactin Levels (IU) Plasma	Pituitary	Organ Weights (mg%) Ovarian	Uterine
Diestrus	0.89	0.28	104	139
Proestrus	0.41	0.20	128	191
Estrus	0.38	0.23	112	204

Uterine tissue weight increased from a mean value of 139 mg% at diestrus to 204 mg% at estrus with 80% of this increase apparent at proestrus. In lesioned animals, the uterine weight remained essentially the same as that of the diestrus control for the first week following the operation. During the second week, the uterine weight increased to a mean of 166 mg% and by the third week after lesions were produced, the uterine weight was 154 mg%.

Plasma levels of prolactin were observed to be higher than pituitary levels in all cases (Fig. 5). At diestrus, the plasma level of prolactin, expressed in international units (IU), was 0.89 IU/ml plasma. This value declined 54% to 0.41 IU/ml plasma during proestrus and was 0.38 IU/ml plasma at estrus. One day following the production of lesions, plasma concentraction of prolactin was 0.88 IU/ml. By one week after lesions, the plasma level of prolactin had decreased 32% to 0.60 IU/ml and by two weeks the value was 0.80 IU/ml, where it remained for

Fig. 4. Effect of basolateral amygdaloid lesions on ovarian and uterine weights. Tissue weight is expressed as mg of tissue per 100 g of body weight (mg%) to correct for body weight variations. Stage of estrous cycle and time following lesioning are plotted along the abscissa. D = diestrus, P = proestrus, E = estrus period in the estrous cycle.

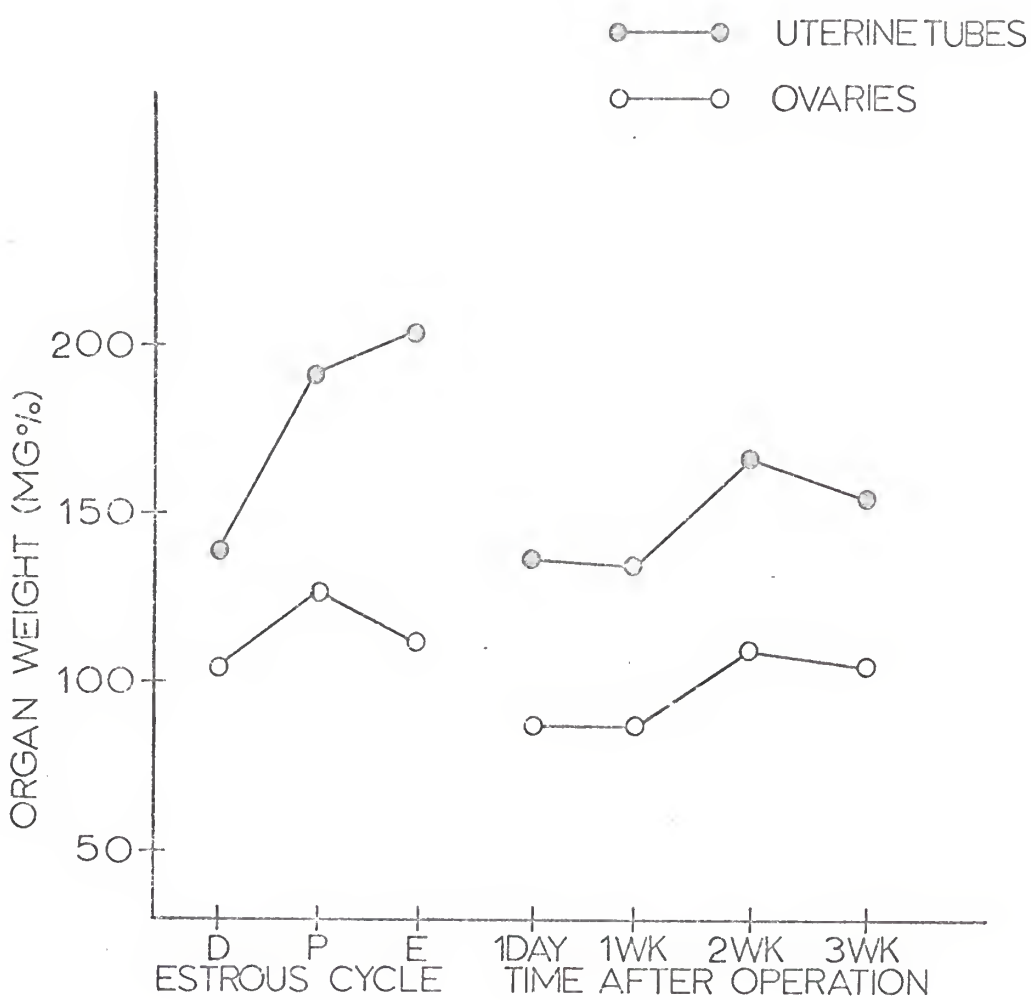
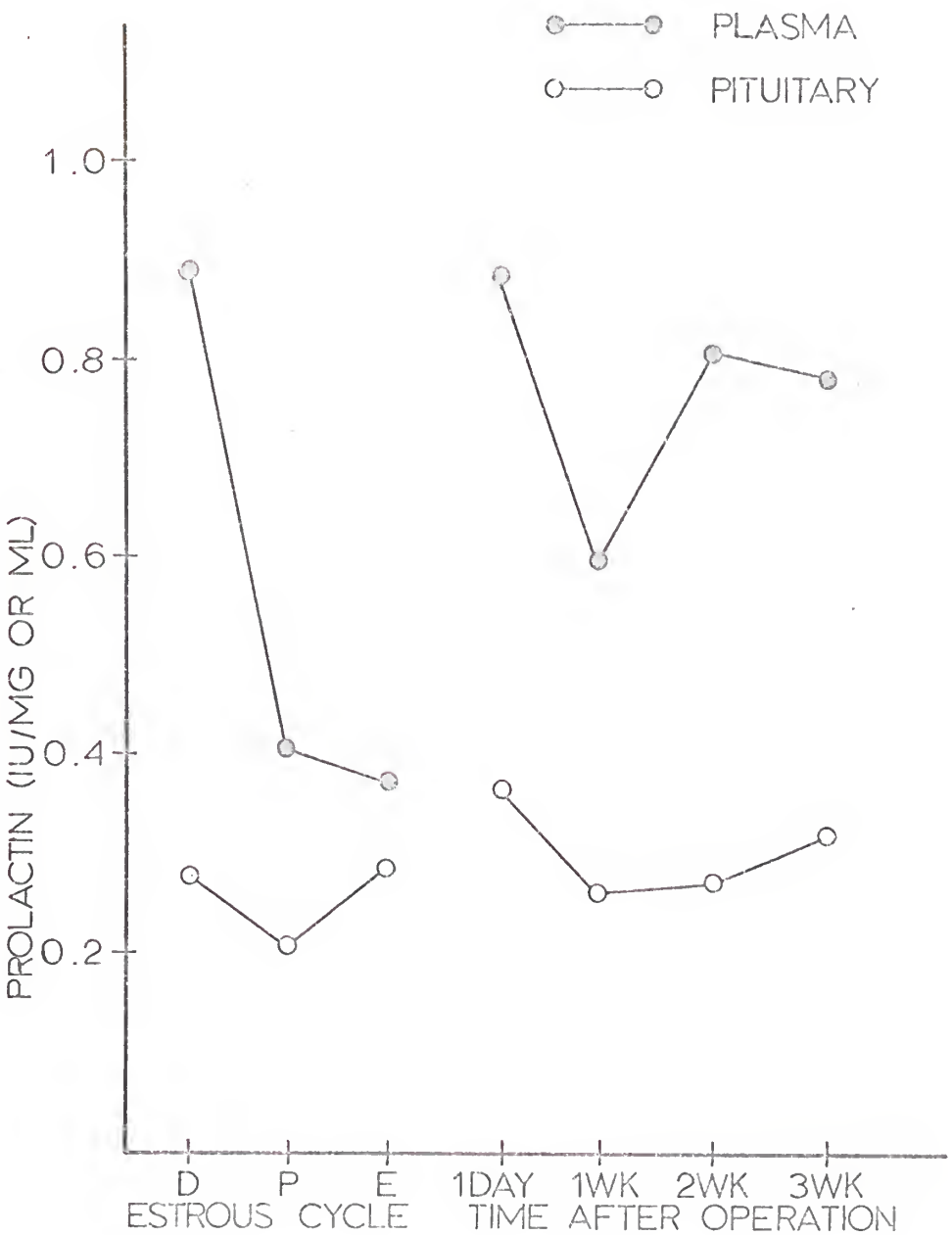

UTERINE TUBES
OVARIES
ORGAN WEIGHT (MG%)
200
150
100
50
D
P
E
ESTROUS CYCLE
1DAY
1WK
2WK
3WK
TIME AFTER OPERATION

Fig. 5. Plasma and pituitary levels of prolactin (IU/mg or ml) during 3 phases of the estrous cycle and various times after basolateral amygdaloid lesions. D = diestrus, P = proestrus, and E = estrus period in the estrous cycle.


PLASMA
PITUITARY
1.0
0.8
0.6
0.4
0.2
PROLACTIN (IU/MG OR ML)
D
P
E
ESTROUS CYCLE
1DAY
1WK
2WK
3WK
TIME AFTER OPERATION

the duration of the experiment.

TABLE II

Effect of amygdaloid lesions on pituitary and plasma prolactin concentrations, and on ovarian and uterine weights.

Time after operation	Prolactin Levels (IU) Plasma	Pituitary	Organ Weights (mg%) Ovarian	Uterine
One day	0.88	0.37	88	137
one week	0.60	0.26	88	136
Two weeks	0.80	0.27	110	166
Three weeks	0.79	0.32	105	154

Pituitary prolactin content of control and experimental animals did not fluctuate as much as plasma content. The diestrus value was 0.28 IU/mg tissue, decreasing 29% to 0.20 IU/mg tissue at proestrus and 0.23 IU/mg tissue by estrus. Values for lesioned animals were somewhat higher than control values with the greatest concentration of prolactin, 0.37 IU/mg tissue at one day following the operation. This value decreased to 0.26 IU/mg tissue at one week and 0.27 IU/mg at two weeks following the operation. A substantial increase to 0.32 IU/mg tissue by the third week post-lesion was observed. Major fluctuations in plasma prolactin levels corresponded to those found in pituitary tissue. Both proestrus and estrus levels for both tissues were less than diestrus levels with the changes in plasma levels being more marked.

DISCUSSION

Pituitary prolactin concentrations of 0.20 IU - 0.36 IU/mg tissue were found to be equivalent with previous levels of 0.10 IU - 0.25 IU/mg tissue determined in the rat (Clark and Baker, 1964; Grosvenor, McCann and Nallar, 1965; Kuroshima, Arimura, Bowers and Schally, 1966; Kurcz, Kovacs, Tiboldi and Orosz, 1967), and 0.10 IU/mg tissue in the goat (Meites and Turner, 1960). Other prolactin levels of pituitary tissue which have been reported are 0.01 IU/mg for the mouse, 0.03 IU/mg for the guinea pig, 0.04 IU/mg for the cow and cat, and 0.02 IU/mg for the rabbit (Meites and Turner, 1950), all of which are lower than the values reported herein.

Recently, Kwa and Verhofstad (1967) developed a sensitive radioimmunoassay for estimation of plasma prolactin concentration. Plasma prolactin levels, using this recently developed assay, were determined in the C_{57}BL x CBA F_1 strain of mice. These values are almost identical with the plasma prolactin levels found in P. m. bairdii. Most reports in the literature can not be compared with the present findings because prolactin usually was measured in animals with tumors or other experimentally induced clinical manifestations.

Abnormal estrous cycles resulting from bilateral ablation of various amygdaloid nuclear groups was theorized to be mediated by increased prolactin secretion (Flerko and Bardos, 1966). This increase in prolactin secretion was thought to induce

formation and maintenance of corpora lutea which produced a "pseudopregnancy-like" prolongation of diestrus. Occasionally, this diestrus was interrupted by short, atypical periods of estrus.

Loss in ovarian weight during the first week after the operation was attributed to absence of corpora lutea due to low plasma prolactin levels at this time. Normally, functional corpora lutea are present at least during the first part of diestrus in long-cycling species, but are absent in short-cycling species such as the rat, mouse and Peromyscus (Muhlbock and Boot, 1956; Nikitovitch-Winer, 1960). This is due to the short duration of the estrous cycle stages which do not allow for sufficient development of a corpus luteum as a result of the effect of LH and prolactin. However, during the second and third weeks following amygdalectomy when plasma prolactin levels had increased significantly over the first week level, ovarian weight also increased substantially, presumably due to corpora lutea formation (Fig. 6). Since pituitary prolactin content did not increase correspondingly with plasma levels during the second and third weeks after lesions, the possibility exists that this hormone was released immediately after synthesis. Ovarian weight during the second week after lesions were placed in the basolateral amygdaloid nucleus was not as great as either proestrus or estrus control values. However, this may have been the result of initial hormonal disturbance, especially of estrogen and progesterone synthesis and release. Therefore, the

Fig. 6. Ovarian section of a female deermouse taken three weeks after lesions were placed in the basolateral amygdaloid nucleus. Note follicles and corpora lutea.

subsequent rise in the secretion of LH and prolactin, both of which can release progesterone, was not sufficient also to induce estrogen secretion which is known to be essential for optimal uterine weight increase. Therefore, any accessory tissue weight increase was probably due only to progesterone secretion as a result of LH and prolactin and was insufficient to give optimal response found during the normal cycle when estrogen is present. The percent weight gain during the second week after lesions was greater than the percent weight gain from diestrus to proestrus, indicating follicle growth, possible ovulation and corpora lutea formation. These corpora lutea were probably maintained by the prolactin since the ovarian weight did not decrease significantly during the third week. This coincides well with the hormonal balance found by Zolovick et al. (1967), where pituitary FSH increased dramatically during the second week. Tonic release of LH at this time would cause ovulation (Koikegami et al., 1954; Shealy and Peele, 1957) and the plasma prolactin levels present would result in corpora lutea formation.

Follicle stimulating hormone values during the first week after the operation remain the same as diestrus values but increase greatly during the second week following lesion production (Zolovick et al., 1967). Uterine growth is initially dependent upon estrogenic steroids which are produced by the ovarian follicle as a result of FSH stimulation. Results bear this out as uterine weight remained at the diestrus control level

during the first week following the operation and increased substantially by the second week. Zolovick et al. (1967) reported that FSH levels dropped markedly during the third week post-lesion; however, uterine weight did not reflect this decrease (Fig. 5). Since ovulation and corpora lutea formation occur during the second week, progesterone production by the corpora lutea seems to be sufficient to maintain uterine weight thereafter.

The resultant decrease in plasma and pituitary prolactin during the first week following the operation possibly might be included in the lethargy phenomenon associated with bilateral ablation of the basolateral amygdaloid complex (Anand and Brobeck, 1952; Schreiner and Kling, 1953; Koidegami et al., 1955; Yamada and Greer, 1960). More likely, the production of lesions in this area might have stimulated adjacent amygdaloid areas or possibly hypothalamic areas which have an inhibitory influence on prolactin secretion. This is a real possibility since there are afferent connections between the amygdala and gonadotropin regulating areas of the hypothalamus (Crosby et al., 1962; Klinger and Gloor, 1955). This would explain the decline in prolactin synthesis and release during the first week after lesions were produced. Amygdaloid areas near the basolateral nucleus which may have a stimulatory influence on hypothalamic production of PIF were very probably stimulated during the electrical discharge used to produce the lesion. Hypothalamic PIF producing areas could also have been stimulated

which would have resulted in further release of PIF, and therefore, decline in prolactin release. Once the effect of this stimulation decreased, prolactin production resumed, seemingly unchanged.

Destruction of the basolateral amygdaloid nucleus did not result in uncontrolled increase or a severe decline in pituitary prolactin production. Therefore, it can be proposed that PIF production by the hypothalamus is not stimulated or inhibited by this area of the amygdala. This is a valid hypothesis since hypothalamic control of the other gonadotropins, FSH and LH is stimulatory rather than inhibitory as it is for prolactin. Another area in the amygdala or other brain areas different from that which influences LH and FSH secretion most probably are involved in control of prolactin secretion.

Neural pathways from the amygdala to hypothalamic areas which regulate gonadotropin secretion are well elucidated (Fox, 1940, 1943; Hall, 1963; Gloor, 1955; Valverde, 1965; Pribram, 1966). Various pathways by which neurosecretory materials can travel from the amygdala to the hypothalamus also have been established (Moore, Shiu-Loong and Heller, 1965). Therefore, if some area of the amygdala exerts an influence on pituitary prolactin secretion via the hypothalamus, ample pathways are present for relaying neural impulses which would effect this control.

Although the hypothalamic inhibition of prolactin secretion is influenced profoundly by circulating levels of estrogens

(Kanematsu and Sawyer, 1963), amygdaloid influence on gonadotropin secretion is not controlled by estrogen feedback (Michael, 1965). Therefore, since hormonal feedback regulation of prolactin via the amygdaloid complex can be excluded, regulation of prolactin secretion is probably derived from sensory input into the amygdala. Auditory, visual and olfactory stimuli affecting flight, fighting, feeding and sexual behavior are modulated by the amygdaloid complex (Goddard, 1964). Lehrman (1964) showed that prolactin secretion can be induced in ring doves by visual observance of nesting behavior without actual participation in this behavior. Presumably, these impulses arising from visual stimulation, are integrated by the phylogenetically older centromedial amygdaloid group because the basolateral complex is relatively undeveloped in non-mammalian phyla (Ursin and Kaada, 1960). The existence of the basolateral complex in mammals undoubtedly influences the phylogenetically older amygdaloid group.

It can be concluded from these data that the basolateral amygdaloid nucleus does not influence prolactin secretion directly. Other amygdaloid areas probably exert some influence on hypothalamic control of gonadotropin secretion; however, neither the area which exerts this control or the mechanism by which this control is effected is known.

Further studies involving stimulation of limbic structures and mechanisms whereby impulses can travel from the amygdala to hypothalamic centers regulating gonadotropin secretion are need-

ed before amygdaloid areas definitely can be implicated in control of pituitary prolactin secretion.

SUMMARY

Bilateral ablation of the basolateral amygdaloid nucleus in Peromyscus maniculatus bairdii resulted in unaltered secretion of prolactin from the pituitary. Although initially, prolactin levels decreased in plasma from 0.88 IU/ml at one day to 0.60 IU/ml at one week following lesioning, and in pituitary tissue from 0.37 IU/mg at one day to 0.26 IU/mg at one week after the operation, these levels returned to near those of diestrus control values by three weeks after the operation. Stimulation, which occured during lesioning and after lesioning until the wound healed, of amygdaloid and hypothalamic areas which inhibit prolactin secretion by increasing hypothalamic production of PIF was considered to cause the initial decrease in prolactin levels. After these effects from lesion production had worn off, normal prolactin secretion was resumed.

Ovarian and uterine weights reflected changes in prolactin levels and were correlated with previous work which estimated FSH and LH levels following bilateral ablation of the basolateral amygdaloid complex as well as with prolactin levels.

Other amygdaloid areas were cited as having some influence on prolactin secretion. However, additional studies are needed to elucidate the brain areas which influence prolactin secretion and mechanisms by which impulses originating from these areas are carried to the hypothalamic gonadotropin regulating centers.

LITERATURE CITED

Anand, B. K. and J. R. Brobeck. (1952). Food intake and spontaneous activity of rats with lesions in the amygdaloid nuclei. J. Neurophysiol. 15:421-430.

Bates, R. W., M. M. Garrison and J. Cornfield. (1963). An improved bio-assay for prolactin using adult pigeons. Endocrinol. 73:217-223.

Chadwick, A. (1963). Detection and assay of prolactin by the local lactogenic response in the rabbit. J. Endocrinol. 27:253-263.

Chen, T. T., R. E. Johnson, W. R. Lyons, C. H. Li and R. D. Cole. (1955). Hormonally induced mammary growth and lactation in the absence of the thyroid. Endocrinol. 57:153-157.

Clark, R. H. and B. L. Baker. (1964). Circadian periodicity in the concentration of prolactin in the rat hypophysis. Science 143:375-376.

Corbin, A. (1966). Pituitary and plasma LH of ovariectomized rats with median eminence implant of LH. Endocrinol. 78:893-896.

Corbin, A. and A. I. Cohen. (1966). Effect of median eminence implants of LH on pituitary LH of female rats. Endocrinol. 78:41-46.

Critchlow, V. and M. E. Bar-Sela. (1967). Control of the onset of puberty. In Neuroendocrinology. Martini, L. and W. F. Ganong, (eds.), Academic Press, New York.

Crosby, E. C., T. Humphrey and E. W. Laurer. (1962). Correlative Anatomy of the Nervous System. MacMillian Co., New York.

Damm, H. C., A. W. Pipes, R. von Berswordt-Wallrabe and C. W. Turner. (1961). Uptake of P^{32} by the pigeon crop-sac as index of lactogenic hormone. Proc. Soc. Exptl. Bio. Med. 108:144-146.

Desclin, L. (1949). A propos de l'action combinee de la prolactine et des oestrogenes sur la structure de l'ovarie chez la rat. Compt. rend. soc. biol. 143:1154-1155.

Desclin, L. (1950). A propos du mecanisme d'action des oestro-

genes surfle lobe anterieur de l'hypophyse chez la rat. Annls. Endocrinol. 11:656-659.

Desclin, L. (1956). Hypothalamus et liberation d'hormone luteotrophique. Experiences de graffe hypophysaire chez la rat hypophysectomise. Action luteotrophe de l'ocytocine. Annls. Endocrinol. 17:586-595.

Egger, M. D. (1967). Responses of hypothalamic neurons to electrical stimulation in the amygdala and hypothalamus. Electroenceph. clin. Neurophysiol. 23:6-15.

Eleftheriou, B. E. and A. J. Zolovick. (1965). The forebrain of the deermouse in stereotaxic coordinates. Kans. Agr. Exp. Sta. Tech. Bull. No. 146.

Eleftheriou, B. E. and A. J. Zolovick. (1966). Effect of amygdaloid lesions on oestrous behavior in the deermouse. J. Reprod. Fertil. 11:451-453.

Eleftheriou, B. E. and A. J. Zolovick. (1967a). Effect of amygdaloid lesions on plasma and pituitary levels of luteinizing hormone. J. Reprod. Fertil. 14:33-37.

Eleftheriou, B. E., A. J. Zolovick and R. L. Norman. (1967b). Effects of amygdaloid lesions on plasma and pituitary levels of luteinizing hormone in the male deermouse. J. Endocrinol. 38:469-474.

Eleftheriou, B. E. (1967c). Effect of amygdaloid nuclear lesions on hypothalamic luteinizing hormone-releasing factor in the male deermouse. J. Endocrinol. 38:479-480.

Evans, A. J. (1962). The *in vitro* effect of prolactin on β-glucuronidase in the testis of the rat. J. Endocrinol. 24:233-244.

Everett, J. W. (1954). Luteotrophic function of autographs of the rat hypophysis. Endocrinol. 54:685-690.

Everett, J. W. (1956). Functional corpora lutea maintained for months by autographs of rat hypophyses. Endocrinol. 58:786-796.

Everett, J. W. and H. M. Radford. (1961). Irritative deposits from stainless steel electrodes in the preoptic rat brain causing release of pituitary gonadotropin. Proc. Soc. Exptl. Bio. Med. 108:604-609.

Flerko, B. and V. Bardos. (1966). Prolongation of dioestrus in rats with diencephalic and mesencephalic lesions. Exptl.

Brain Res. 1:299-305.

Fonberg, E. (1963). The inhibitory role of amygdala stimulation. Acta Biolog. Experimentalia 23:171-180.

Fonberg, E. and J. M. R. Delgado, (1961). Avoidance and alimentary reactions during amygdala stimulation. J. Neurophysiol. 24:651-664.

Fox, C. A. (1940). Certain basal telencephalic centers in the cat. J. Comp. Neurol. 72:1-40.

Fox, C. A. (1943). The stria terminalis, longitudinal association bundle and precommissural fornix fibers in the cat. J. Comp. Neurol. 79:277-295.

Frost, L., M. Baldwin and C. Wood. (1958). Investigation of the primate amygdala: Movement of the face and jaws. Neurology 8:543-546.

Gloor, P. (1955). Electrophysiological studies on the connections of the amygdaloid nucleus in the cat. Part II: The electrophysiological properties of the amygdaloid projection system. Electroenceph. clin. Neurophysiol. 7:243-264.

Goddard, G. V. (1964). Functions of the amygdala. Psychol. Bull. 62:89-109.

Grosvenor, C. E. and C. W. Turner. (1958). Assay of lactogenic hormone. Endocrinol. 63:530-534.

Grosvenor, C. E., S. M. McCann and R. Nallar. (1965). Inhibition of nursing-induced and stress-induced fall in pituitary prolactin concentration in lactating rats by injection of acid extracts of bovine hypothalamus. Endocrinol. 76:883-889.

Hall, E. A. (1963). Efferent connection of the basal and lateral nuclei of the amygdala in the cat. Amer. J. Anat. 113:139-151.

Haun, C. K. and C. H. Sawyer. (1960). Initiation of lactation in rabbits following placement of hypothalamic lesions. Endocrinol. 67:270-272.

Kanematsu, S. and C. H. Sawyer. (1963). Effects of intrahypothalamic and intrahypophysial estrogen implants on pituitary prolactin and lactation in the rabbit. Endocrinol. 72:243-252.

Kanematsu, S., J. Hillard and C. H. Sawyer. (1963). Effect of hypothalamic lesions on pituitary prolactin content in the

rabbit. Endocrinol. 73:345-348.

Kling, A. (1965). Behavorial and somatic development following lesions of the amygdala in the cat. J. Psychiat. Res. 3:263-273.

Kling, A., J. Orbach, N. B. Schwartz and J. C. Towne. (1960). Injury to the limbic system and associated structures in the cat. Arch. gen. Psychiat. 3:391-420.

Klinger, J. and P. Gloor. (1960). The connections of the amygdala and of the anterior temporal cortex in the human brain. J. Comp. Neurol. 115:333-369.

Koikegami, H., T. Yamada and K. Usui. (1954). Stimulation of amygdaloid nuclei and periamygdaloid cortex with special reference to its effects on uterine movement and ovulation. Folia Psychiat. Neurol. Japan. 8:7-36.

Koikegami, H., S. Fuse, T. Yokoyama, T. Watanabe and H. Watanabe. (1955). Contributions to the comparative anatomy of the amygdaloid nuclei of mammals with some experiments of their destruction or stimulation. Folia Psychiat. neurol. Japon. 7:86-103.

Kovacic, N. (1962). Prolongation of dioestrus in the mouse as a quantitative assay of the luteotrophic activity of prolactin. J. Endocrinol. 24:227-231.

Kurcz, M., K. Kovacs, T. Tiboldi and A. Orosz. (1967). Effects of androgenisation on adenohypophyseal prolactin content in rats. Acta Endocrinol. 54:663-667.

Kuroshima, A., A. Arimura, C. Y. Bowers and A. V. Schally. (1966). Inhibition by pig hypothalamic extracts of depletion of pituitary prolactin in rats following cervical stimulation. Endocrinol. 78:216-217.

Kwa, H. G. and F. Verhofstad. (1967). Prolactin levels in the plasma of female (C_{57}BL x CBA) F_1 mice. J. Endocrinol. 38:81-82.

Lehrman, Daniel S. (1964). The reproductive behavior of ring doves. Scientific American 211:48-54.

Lyons, W. R. (1937). Preparation and assay of mammatropic hormone. Proc. Soc. Exptl. Bio. Med. 35:645-648.

Lyons, W. R. (1958). Hormonal synergism in mammary growth. Proc. Roy. Soc., London, ser. B. 149:303-325.

Lyons, W. R., C. H. Li, R. D. Cole and R. S. Johnson. (1953). Some of the hormones required by the mammary gland in its development and function. J. Clin. Endocrinol. 13:836-837.

Masserman, J. H., M. Levitt, T. McAvog, A. Kling and C. Pechtel. (1958). The amygdalae and Behavior. Amer. J. Psychiat. 115:14-17.

Meites, J. and C. W. Turner. (1950). Lactogenic hormones. In Hormone Assay. Emmens, C. W. (ed.), Academic Press, New York.

Meites, J., C. S. Nicoll and P. K. Talwalker. (1963). The central nervous system and the secretion and release of prolactin. In Advances in Neuroendocrinology. Nalbandov, A. V. (ed.), Univ. Illinois Press, Urbana.

Michael, R. P. (1965). Neurological mechanisms and the control of sexual behavior. Ann. Rev. Sc. Basis Med. 19:316-333.

Mixner, J. P., A. A. Lewis and C. W. Turner. (1940). Evidence for the prescence of a second mammogenic (lobule-alveolar) factor in the anterior pituitary. Endocrinol. 27:888-892.

Moore, R. Y., R. W. Shiu-Loong and A. Heller. (1965). Regional effects of hypothalamic lesions on brain serotonin. Arch. Neurol. 13:346-354.

Muhlbock, O. and L. M. Boot. (1956). La fonction hormonale d'ovaries, testicles et hypophyses transplantes chez des souches de souris genetiquement suies. Annls. Endocrinol. 17:338-343.

Nikitovitch-Winer, M. (1960). The influence of the hypothalamus on luteotropin secretion in the rat. Mem. Soc. Endocrinol. 9:70-72.

Nikitovitch-Winer, M. (1965). Effect of hypophysial stalk transection on luteotropic hormone secretion in the rat. Endocrinol. 77:658-666.

Nikitovitch-Winer, M. and J. W. Everett. (1958). Comparative study of luteotropin secretion by hypophysial autotransplants in the rat. Effects of site and stages of the estrous cycle. Endocrinol. 62:522-532.

Powers, M. M. and G. Clark. (1955). An evaluation of cresyl echt violet acetate as a nissel stain. Stain Techn. 30: 83-92.

Pribram, K. H. (1966). The neurobehavorial analysis of the

limbic forebrain mechanisms. In Advances in the study of Behavior. Lehrman, D. S., R. A. Hinde and E. Shaw (eds.), (in press).

Riddle, O., R. W. Bates and S. W. Dykshorn. (1933). The preparation, identification and assay of prolactin - a hormone of the anterior pituitary. Amer. J. Physiol. 105:191-216.

Rothchild, I. (1960). The corpus luteum-pituitary relationship: The association between the cause of luteotrophin secretion and the cause of follicular quiescence during lactation: The basis for a tentative theory of the corpus luteum-pituitary relationship in the rat. Endocrinol. 67:9-41.

Schally, A. V., J. Meites, C. Y. Bowers and A. Ratner. (1964). Identity of prolactin inhibiting factor (PIF) and luteinizing hormone-releasing factor (LRF). Proc. Soc. Exptl. Bio. Med. 117:252-254.

Schreiner, L. and A. Kling. (1953). Behavorial changes following rhinecephalic injury in cats. J. Neurophysiol. 16: 643-659.

Schwartz, N. B. and A. Kling. (1964). The effect of amygdaloid lesions on feeding, grooming and reproduction in rats. Acta. Neurovegetativa 26:12-16.

Shealy, C. N. and T. L. Peele. (1957). Studies on the amygdaloid nucleus of the cat. J. Neurophysiol. 20:125-139.

Simkin, B. and D. Goodart. (1960). Preliminary observations on prolactin activity in human blood. J. Clin. Endocrinol. 20:1095-1106.

Strickler, P. and Grueter, F. (1928). Action du lobe anterieur de l'hypophyse sur la montee laiteuse. Comp. rend soc. biol. 99:1978-1980.

Strickler, P. and Grueter, F. (1929). Recherches experimentales sur les fonctions du lobe anterieur de l'hypophyse: Influence des extraits du lobe anterieur sur l'appareil genital de la lapine et sur la montee laiteuse. Presse med. 37:1268-1271.

Ursin, H. (1960). The temporal lobe structure of fear and anger. Acta Psychiat. scand. 35:378-396.

Ursin, H. and B. R. Kaada. (1960). Functional localization within the amygdaloid complex in the cat. Electroenceph. clin. Neurophysiol. 12:1-20.

Valverde, F. (1965). Studies on the piriform lobe. Harvard Univ. Press, Cambridge, Mass.

Walker, A. E., A. F. Thompson and J. D. McQueen. (1953). Behavior and the temporal rhinecephalon in the monkey. Johns Hopkins Bull. 93:65-93.

Whitfield, I. C. (1964). Manual of Experimental Electrophysiology. Macmillian Co., New York.

Wolthius, O. L. (1963). A new prolactin assay method: Some experiments which provide arguments for its specificity. Acta Endocrinol. 42:380-388.

Wood, C. D. (1958). Behavorial changes following discrete lesions of temporal lobe structures. Neurology 8:215-220.

Wood, C. D., B. Schottelius, L. Frost and M. Baldwin. (1958). Localization within the amygdaloid complex of anesthetized animals. Neurology 8:477-480.

Yamada, T. and M. A. Greer. (1960). The effect of bilateral ablation of the amygdala on endocrine function in the rat. Endocrinol. 66:565-574.

Zolovick, A. J., R. L. Norman and B. E. Eleftheriou. (1967). Effect of amygdaloid lesions on follicle stimulating hormone secretion in the deermouse. (unpublished observations).

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EFFECT OF BASOLATERAL AMYGDALOID LESIONS ON PROLACTIN SECRETION IN *PEROMYSCUS MANICULATUS BAIRDII*

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Bilateral lesions produced by electrocoagulation in the basolateral amygdaloid nuclear complex were found to induce non-significant affects in hypophyseal and plasma levels of prolactin and produce sex accessory tissue changes in the female deermouse (Peromyscus maniculatus bairdii).

Prolactin was measured quantitatively in plasma and pituitary tissue during diestrus, proestrus and estrus and at one day, one, two and three weeks following stereotaxic placement of electrolytic lesions in the basolateral amygdaloid nuclear complex. Ovarian and uterine weights and histological examinations were made to provide additional parameters by which prolactin effects could be evaluated. Prolactin values for diestrus, proestrus and estrus controls were comparable to those found previously by other investigators in the mouse. Decreased pituitary and plasma prolactin levels were observed at one week following the operation. This depletion was attributed to stimulation, at the time of lesioning and during healing of the wound, of other amygdaloid and hypothalamic areas which may have a stimulatory influence on the production of prolactin-inhibiting factor (PIF). Decreased ovarian and uterine weights reflected low prolactin levels, and probably low levels of other gonadotropins, during the initial week after the operation. During the second and third weeks after the operation, prolactin levels increased from 0.60 IU/ml to 0.80 IU/ml in plasma and from 0.26 IU/mg to 0.32 IU/mg in pituitary tissue. The latter values for plasma and pituitary tissue were comparable to values

for diestrus controls. This increase in pituitary and plasma levels of prolactin was reflected by increase in both ovarian and uterine weights for this period. Although the ovaries and uterine tubes are not dependent primarily on prolactin influence for growth, corpora lutea maintenance which increases ovarian weight directly and maintains uterine weight indirectly through progesterone secretion, is intimately dependent on prolactin activity. Follicle stimulating hormone and luteinizing hormone levels determined in previous experiments were also correlated with ovarian and uterine weights.

Prolactin secretion by the pituitary was not altered significantly by bilateral destruction of the basolateral amygdaloid nucleus. This would indicate that this area of the amygdala does not influence hypothalamic inhibition or stimulation of prolactin secretion. Evidence was cited for the involvement of other amygdaloid areas in the control of gonadotropin secretion. However, no experimental data are available which directly implicate specific areas involved in this control or the means by which this control is effected.

Additional experimentation is necessary for elucidation of processes by which the amygdala might exert some influence on hypothalamic inhibition of pituitary secretion of prolactin and the pathways by which this influence is implemented.