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The Cortical Nucleus of the Amygdala as a Component in the Systems Regulating Gonadotrophin Secretion

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THE CORTICAL NUCLEUS OF THE AMYGDALA AS A COMPONENT IN THE SYSTEMS REGULATING GONADOTROPHIN SECRETION

by

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BIOGRAPHY

Stuart W. Smith was born on July 25, 1943, in Chicago, Illinois. He graduated from Amundson High School and continued his studies at MacMurray College in Jacksonville, Illinois, and at Michigan State University, East Lansing, Michigan. He received the Bachelor of Arts degree in Physics from MacMurray College in May, 1966.

Graduate training in the Department of Physiology was begun in the summer of 1966, and he began his dissertation work with Dr. Irene Lawton in 1967. He was supported by an NDEA - Title IV Fellowship from 1966 to 1969 and by a Research Training Grant of the National Institutes of General Medical Sciences of the National Institutes of Health, from 1969 to 1970.

Mr. Smith was married on August 19, 1967, to Lynda, and they have a son, Martin Everett, who was born on December 9, 1969.

ABSTRACT

The hypothalamus has been shown to be intimately involved in the control of ovulation and the estrous cycle by many experimental approaches. Evidence for regulative influences by extrahypothalamic brain structures has also been accumulating. In as much as the amygdala seems to have some influence on gonadotrophin secretion in various types of non-cyclic animals, the importance of the amygdala on gonadotrophin secretion in cyclic animals was investigated.

Rats of proven cyclicity (vaginal smears) had bilateral electrolytic lesions placed in the cortical nucleus of the amygdala during metestrus. Animals were autopsied 1) during the third cycle between 2:30 PM and 3:30 PM on proestrus, 10:00 AM and 11:00 AM, or 2:30-3:30 PM on estrus, and between 2:30 PM and 3:30 PM on metestrus of the fourth cycle, 2) around the 60th day postoperative between 2:30-3:30 PM on proestrus, and between 10:00 and 11:00 AM or between 2:30-3:30 PM on estrus, 3) around the 120th day postoperative between 2:30-3:30 PM on proestrus and between 2:30-3:30 PM on estrus. Neither body nor organ weights (pituitary, thyroid, adrenal, ovarian, and uterine) developed any trend following lesions to deviate from values in either control or sham lesioned (electrode lowered but no current) animals. Ovulation, as indicated by the number of ova in the oviduct, was not altered by lesioning. Ovarian histology of ovaries from animals in the three cycle study reveals several corpora lutea per lesioned animal on metestrus have a prominent lumen; control animals have solid corpora lutea by metestrus. Lesioned animals from the 2-month and 4-month studies had luteal lumens during estrus, but so did their respective

control animals. No consistent effect was noted by the lesion on plasma LH in the 3-cycle study; however, there was some indication the afternoon levels of LH may have been slightly elevated on estrus, but not during any of the other autopsy time periods. Plasma LH showed no apparent alterations in the 2-month study. Plasma LH measurements in the 4-month study indicate the proestrus critical period may be advanced. Pituitary LH measurements in the 3-cycle study reveal an increased reaccumulation of pituitary LH storage following ovulation in lesioned animals. Pituitary LH measurement from the 2- and 4-month studies show no difference between lesioned and control animals because these older control animals had higher LH stores than their younger experimental counterparts. It appears from these data the ACO may have an effective inhibitory influence in cyclic animals but much attenuated from the role indicated from non-cyclic animals. Because the ACO lesioned animals appear in some respects similar to older animals, the loss of certain amygdaloid influences as animals age may be involved in some of the endocrine alterations in aged animals. Evaluation of FSH regulation in ACO lesioned animals was tested by the ovarian compensatory hypertrophy (OCH) response. Animals were lesioned and unilaterally ovariectomized the same day, then autopsied ten days later. Lesions of the ACO or of the stria terminalis prevented the OCH response, sham lesions or cerebral cortex lesions did not prevent the OCH response. Since the age of the animals in the OCH study and the 3-cycle study were comparable, it appears the ACO lesions may affect the LH and FSH regulatory neuroendocrine networks in the opposite fashions, i.e., augmenting LH secretion while depressing FSH secretion.

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Chapter I

LITERATURE REVIEW

The central nervous system (CNS) plays a primary role in maintaining the rhythmicity of the estrous cycle. This regulatory role of the CNS can be modulated by both the ovarian and the pituitary hormones. The prominent CNS regulation appears to reside within the hypothalamus in that an independent functional hierarchy has been revealed in this region. The basal hypothalamus will maintain ovarian histology but not ovulation. Deafferentation of the anterior hypothalamus-preoptic area (POA) as a unit with the basal hypothalamus will support both ovarian histology and ovulation, but the ovulations are at longer intervals than normal. The hypothalamus thus has the ability to support ovulation but requires extra hypothalamic influences to maintain uniform cyclicity of four- or five-day cycles characteristic of the rat. It is the purpose of the literature review to explore functional roles of the ovary, pituitary, and hypothalamus in the mechanics of the estrous cycle as the background against which the role that the amygdala may play can be discussed, with particular emphasis placed on the cortical nucleus of the amygdala.

A. Gross Anatomical Changes Associated with the Stages of the Rat Estrous Cycle

As discussed below, changes in organ cytology and in weight or size are associated with the estrous cycle. These changes reveal fluctuation of hormones responsible for maintaining the estrous cycle.

1. Vaginal Cytology

Efforts to elucidate the female reproductive cycle were of limited value until 1917 when Stockard and Papanicolaou (263) reported their success in identifying the stages of the guinea-pig estrous cycle by making daily examinations of the cell types within the vagina. Applying this newly discovered technique, Long and Evans (189) published results of an intensive investigation on the rat, correlating the periodic anatomical changes of the reproductive tract with the different cell types obtained from the vagina. Their investigations revealed a predominance of four- to five-day cyclic patterns recognizable from the periodical occurrence of leukocytic and cornified epithelial cells in the vagina (189). Placing the rats in an environment of controlled daily lighting, Everett (86, 89) described the cytological changes as a daily sequence during the cycle which was recognized as being exactly four or five days long (see also section I.D.2.). In describing the daily cyclic changes, the names suggested by Long and Evans (189) for the cyclic stages will be retained throughout the dissertation.

The Four-day Cycle:

METESTRUS - the first day of the cycle is usually characterized by large numbers of leukocytic cells. It is not unusual, though, for variable numbers of cornified cells to be interspersed between the leukocytes.

DIESTRUS - the second day of the cycle is characterized by a much less dense accumulation of cell types. Although leukocytes often predominate, cornified and nucleated epithelial cells are frequently as numerous as the leukocytes.

PROESTRUS - the third day of the cycle is characterized by very few or no leukocytes. Either nucleated or cornified epithelial cells or a combination of these two predominate.

ESTRUS - the fourth day of the cycle is characterized by very large numbers of cornified epithelial cells, occasionally a few nucleated epithelial cells, and virtually no leukocytes.

The Five-day Cycle:

The five-day cycle has an extra day of diestrus. Like the first, the second day is characterized by roughly equal numbers of leukocytes, nucleated epithelial, and cornified epithelial cells. Thus, proestrus and estrus are the fourth and fifth days of the five-day cycle, respectively, with the same cytological characteristics seen for the four-day cycle.

Internal physiological phenomena, as discussed below, were found coincident with specific days of t he vaginal cycle. The correlation of these phenomena has been a driving force toward unravelling the complex interactions comprising the rat estrous cycle.

2. Ovarian Cyclicity

Examination of ovaries from animals during the various days of the cycle reveals that maturation of the primordial follicles begins during metestrus; they ripen on through diestrus reaching maturity during proestrus with ovulation occurring during estrus (172). After ovulation, the follicular granulosal cells proliferate forming the corpora lutea (30, 189). Although the corpora lutea persist for several cycles in the rat (189), they are apparently functional for less than one cycle (86, 189).

3. Ovulation

The presence of eggs in the oviduct affords direct evidence that ovulation has occurred. In rats with four-day cycles, ovulation begins as early as 1:10 AM and is completed by 2:30 AM on the day of estrus with rats under a lighting regime of lights on from 5:00 AM until 7:00 PM (86). In a recent study on four-day rats under the same lighting regimen (118) ovulation occurred

later, i.e., earliest eggs found at 2:30 AM with ovulation completed by 4:00 AM on estrus. These differences may reflect strain differences of rat colonies used.

4. Uterine Cyclicity

Long and Evans (189) first reported the accumulation of fluid within the lumen of the uterine horns of the rat at the stages of the estrous cycle characterized by cornified and/or nucleated epithelial cells in the vaginal smear. Subsequent investigators confined the distention of the uterus to proestrus (13, 248, 274). During the estrous cycle uterine weight fluctuates significantly such that the maximum weight is found at proestrus (13, 148, 248). Much of this weight gain is due to an increase of tissue fluid, although a significant increase in tissue mass also occurs (13, 248). By estrus, uterine weight has declined to a significantly lower weight than that of proestrus; by metestrus an additional drop has occurred, although not necessarily significantly lower than that of estrus (236, 248).

B. The Ovary as an Endocrine Organ

Removal of both ovaries results in atrophy of the female genital tract while autotransplantation of the ovaries prevents it (189). Likewise, ovariectomy soon after coitus and before implantation of the embryo results in loss of the embryo and atrophy of the genital tract; however, if removal of the ovaries is delayed until after implantation, degeneration does not occur and the pregnancy results in a normal birth (49).

1. Endocrine Function of the Follicle

Allen and Doisy (3) made the first successful demonstration of an ovarian extract capable of sustaining the physiological phenomena associated with the estrous state of the female. Noting that follicles were always associated with the stage of the cycle when uterine growth and vaginal cell proliferation were

evident (2), it was assumed that the follicles were elaborating an estrus-inducing hormone. With injections of follicular fluid, aspirated from the 1 largest follicles of cow and pig ovaries, into ovariectomized rats or mice, vaginal cornification, maintenance of uterine structure, accumulation of uterine lumenal fluid, and mating behavior were demonstrated (3, 4). Subsequently, chemicals were isolated (193) and identified (68) from the follicular liquor which could induce estrus in ovariectomized animals.

2. Endocrine Function of the Corpus Luteum

Although corpora lutea were suspected of having an endocrine function related to implantation and the initial stages of pregnancy (188), researchers were requiring of luteal extracts an end result which would mimic the characteristics of estrus (3). After the demonstration by Allen and Doisy (3) that the hormone(s) responsible for estrus originated from the follicles, re-evaluation of the endocrine function of the corpora lutea led to the discovery of a luteal extract, distinct from the follicular extract, necessary for nidation and the early stage of pregnancy (5, 49, 51). Soon afterwards, compounds which had "progestational" properties were isolated (275) from extracts and identified (6).

C. Pituitary - Ovarian Axis

Although early research on ovarian cyclicity tended to implicate an ovarian "autoregulatory" mechanism maintaining the estrous cycle (188), there were suggestive data indicating extra-ovarian influences involved in the estrous cycle (183). Removal of one ovary was found to result in hypertrophy of the remaining ovary (43); this phenomenon is so regular that it has been termed "the law of follicular constancy" (183). Ovarian transplant studies also yielded evidence for extra-ovarian regulation (183, 189, 263). Transplantation of ovaries into castrated males results only in the development of

follicles (183, 263), whereas corpora lutea develop if the ovary is transplanted into an ovariectomized female (183, 189). Thus, under the influence of the male milieu interne the ovary does not cycle. Furthermore, Long and Evans (80) demonstrated that ovaries from immature rats transplanted into ovariectomized adult females supported reproductive cyclicity, whereas ovaries from adult rats transplanted into immature ovariectomized rats did not support cyclicity. Therefore, the milieu interne of the recipient animal had directed the functional development of the donor ovary. These studies strongly argued against the estrous cycle being of independent ovarian origin and provided an impetus for subsequent research on the locus of higher levels of ovarian regulation.

The first direct indication that ovarian function could be influenced by the pituitary came with the demonstration that daily injections of pituitary extracts could delay the onset of puberty and disturb the estrous cycle (79, 80). Although reproductive cyclicity was suppressed, large ovaries containing both corpora lutea and follicles, and normal appearing uteri, were found (80). An hypophyseal influence over ovarian function was confirmed by P. E. Smith (257); a consistent and reproducible acceleration of sexual maturity was found following injection of pituitary extracts into immature rats. Removal of the ovaries prior to treatment prevented the precocious development. Using the same techniques, mating was noted in young female mice in which precocious development had been induced, indicating a true estrus had been achieved (260). The introduction of a technique by which one could hypophysectomize an animal without producing damage to the close lying brain tissue was a marked methodological advancement (258), since there was some argument at that time over the importance of neural tissue (see section I.D.). Ablation of the pituitary without damage to the central nervous system (CNS) resulted in atrophy of the

reproductive system and subsequent cessation of estrous cycles; daily injections of extracts of fresh pituitaries resulted in repair of the reproductive system (258). Hypophysectomy of immature rats prevented vaginal opening; however, following injections of pituitary extracts, vaginal opening did occur (259). Degeneration of accessory sex tissues in ovariectomized animals could not be prevented by injections of fresh pituitaries (259). An important ovarian dependence on pituitary secretion(s) thus seemed evident (259).

1. Isolation of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH)

Evidence for two factors in crude extracts of the pituitary was presented in 1928 (81); one had profound effects on somatic growth, although apparently was also capable of inducing moderate luteinization of follicles, the other could induce precocious puberty. The authors felt the luteinizing ability of the former extract was secondary to its primary growth-promoting effect and was therefore not of major importance. That the pituitary did contain two substances, rather than one, having a profound influence over ovarian physiology was demonstrated be Fevold, Hisaw, and Lenard in 1931 (99). These substances were termed follicle stimulating hormone (FSH) and luteinizing hormone (LH). Although purification was not achieved at that time, the compounds were shown to be chemically separable and distinct. They were also physiologically unique; the follicle stimulator only induced follicular growth, whereas the luteinizer failed in this respect. It would induce formation of corpora lutea from the previously developed follicles. These substances were subsequently isolated in crystalline form, the luteinizer (LH) in 1940 (178) and the follicle stimulator (FSH) in 1949 (179).

2. Some Physiologic Effects of FSH and LH

When first reported (99), LH and FSH were distinguished as separate

entities because of specific actions on ovarian development which paralleled the changes occurring in the ovary during the estrous cycle, i.e., follicular development and luteinization of developed follicles. However, more subtle differences were soon discovered.

a. Estrogen secretion

Fevold (98) reported estrogen secretion by the ovary to be stimulated by a combined action of FSH and LH. While relatively high doses of FSH alone had a marginal ability to stimulate estrogen secretion (measured indirectly using an increase in uterine weight as an end point), estrogen secretion was markedly elevated by adding minute amounts of LH; LH alone, even at very high doses, did not induce estrogen secretion (as measured by uterine weights). Injection of smaller quantities of FSH failed to induce estrogen secretion unless accompanied by minute quantities of LH. Thus, divergent roles for the two pituitary compounds become apparent. Since the FSH preparation was known not to be pure, LH contamination may have been responsible for the slight secretion of estrogen following the high doses of FSH alone. It was suggested that pure FSH may not stimulate estrogen secretion at all, but rather only stimulate follicular growth. LH, on the other hand, although unable to induce estrogen secretion by itself, appeared to synergize with subthreshold doses of FSH to induce estrogen secretion. Using a highly purified FSH and LH, Lostroh and Johnson (190) confirmed and extended these results. Fevold's suspicion that pure FSH alone might be ineffective in inducing estrogen secretion was verified. A critical minimal level of FSH (about 1 μg/day) was shown to be needed for both follicular development and promotion of estrogen secretion; however, with FSH below this minimal dosage, LH could induce a dose-response curve for estrogen secretion, thus confirming the highly sensitive synergistic relationship between LH and FSH.

b. Ovulation

Immature rat ovarian follicles ripened by exogenous hormone treatments can be induced to ovulate by LH and/or FSH (119, 190). Even though FSH alone can induce ovulation, other events following ovulation are abnormal; the uterus remains ballooned the next day and the corpora lutea are not well developed. Ovulation induced by a mixture of FSH and LH, or with just LH, is not accompanied by these abnormalities (119). Since both FSH (202) and LH (250) are elevated before ovulation both hormones may play a physiologic role. Thus, LH may be the primary hormone inducing the physiological events associated with ovulation, while FSH plays a secondary role synergizing with LH to produce a maximal ovulatory stimulus.

3. General Ovarian Hormone Influence over Pituitary Function in Female Rats

Evidence for ovarian hormone involvement in the regulation of pituitary gonadotrophin levels was first provided by the discovery that pituitaries from gonadectomized rats had greater growth-promoting effects on the ovaries of immature rats than pituitaries from intact rats (78, 82). Significantly, the longer the animals were without ovaries, the more potent were their pituitaries in promoting the growth of the immature recipient animals' ovaries (82). The increased pituitary gonadotropjin potency of gonadectomized rats was soon shown to be inhibited by estrogenic substances (206). Moore and Price presented a formal hypothesis of inhibition of pituitary secretion of gonadotrophin by the gonadal hormones in an elegant series of experiments (213). It was demonstrated that gonadal extracts could induce degenerative changes in the gonads but these changes could be prevented if extracts of either pituitaries or human pregnancy urine (known to have gonadotrophin-like effects) were administered simultaneously with the gonadal extract. This hypothesis was termed "push-pull" in that the pituitary secretions would push the ovary into secreting

its hormones but, in return, the ovarian hormones would pull back on, or reduce, the pituitary secretions (50). Although this concept gained wide acceptance, there was some controversy over its completeness as an explanation of the phenomena associated with the ovarian-pituitary axis. Objection was raised by Heller, Heller, and Severinghaus (142), who could induce the well known ovarian compensatory hypertrophy (43) and prevent the hypertrophy by injections of estrogenic substances, but found that estrogen did not prevent the accumulation of pituitary gonadotrophin after unilateral ovariectomy. Since the pituitary contains several gonadotrophins, a more important question than what happens to the total gonadotrophin content of the pituitary is the question of what the ovarian hormones do to the individual gonadotrophins. An answer to this question was attempted by Byrnes and Meyer (37). The index of pituitary gonadotrophin secretion (ovarian weight) was found to oscillate around the normal average weight, such that at a dose of .02 µg and .1 µg of estradiol, ovarian weight was low but at .08 µg ovarian weight was elevated. They concluded that whereas FSH secretion appeared to be inhibited by both low and higher circulating estrogen levels, LH secretion appeared to be stimulated by low estrogen levels but inhibited by high levels of estrogen.

With the advent of specific assays for gonadotrophin, detailed studies of FSH and LH could be made (211, 224, 261).

a. Pituitary and plasma LH and FSH in the absence of ovarian steroids

Bilateral ovariectomy of immature and mature rats results in the elevation of both pituitary and plasma LH (227, 234, 267). This elevation has been reported to persist as long as one year after ovariectomy (227). Pituitary (54, 114, 227) and plasma (53) FSH also are elevated following ovariectomy. Species differences appear to exist, however, since in the mouse pituitary and plasma FSH rise after ovariectomy but pituitary and plasma LH do not (227).

b. Plasma and pituitary LH and FSH in the presence of ovarian steroids

1) Estrogen

Estrogen injections will prevent the post-castration rise in both plasma and pituitary LH (115, 226, 234, 267). Although Parlow (226) found that FSH also was not detectable in plasma from estrogen-treated ovariectomized rats, a differential responsiveness of FSH and LH to the inhibitory action of estrogen in ovariectomized rats was noted. Injections of physiological doses of estrogen (0.4 µg/day), which prevented the post-ovariectomy rise in pituitary LH, failed to prevent the rise of FSH. Even with a dose of 2.0 µg/day, which is beyond the physiological range (113, 226), only a marginally significant ($p = .05$) drop in pituitary FSH was noted. Therefore, it appears that the FSH regulatory system is not as sensitive to the inhibitory effects of estrogen as is the LH system.

Ovarian compensatory hypertrophy (OCH) following unilateral ovariectomy was felt to be due to increased circulating gonadotrophin levels (142). However, no elevation of LH or FSH was detectable in rat plasma (71). Using a more sensitive modification of the Steelman-Pohley FSH assay (261), Benson et al. (24) were able to detect a transient, but significant, rise in plasma FSH on the fourth day after unilateral ovariectomy, with a return to lower levels after about two weeks. Estrogen inhibits both the OCH response (24, 142) and the transient rise in plasma FSH (24).

In addition to these inhibitory effects of estrogen, estrogen also has a facilitative influence on gonadotrophin secretion. Thus, an injection on the second day of a five-day cycle advances ovulation by one day making the cycle four days long (86). Estrogen can also induce ovulation in the rabbit (242) and pregnant rat (85). The direct effect of estrogen on LH has revealed that plasma LH can be elevated following daily injection of estradiol for seven

days (39) or on the third day following a single injection of estrogen (19).

2) Progesterone

It has been long known that progesterone is less effective than estrogen in altering pituitary gonadotrophin content (126). Confirming this, and detailing the specific effect of progesterone on LH, McCann (196) found progesterone to have only very minimal effects on the inhibition of LH release in ovariectomized rats when physiological levels (83, 192) of 5 mg/day for three days were given; only when the dose was increased to 25 mg/day for two days was LH release suppressed. However, pretreatment of the ovariectomized rats with estrodiol (1 µg/day) for two days sensitized the animals so that physiological levels of progesterone (4 mg/day for three days) were now effective in depressing the elevated LH levels (196). These results were partially confirmed by Nallar et al. (215) who reported that 1.5 mg progesterone for four days failed to reduce plasma LH in ovariectomized rats.

Daily injections of progesterone (.05 IU/day for twenty days) in guinea-pigs prevents ovulation but does not interfere with follicular development; therefore progesterone, while inhibiting the ovulatory levels of gonadotrophins, does not inhibit the follicular growth-promoting levels of FSH (and IH) (61). The same results seem applicable during pregnancy (61). However, progesterone (1 mg/day) is apparently capable of inhibiting the transient increase of FSH starting four days after unilateral ovariectomy (24).

In addition to its inhibitory effects, progesterone also has facilitative influences over gonadotrophin secretion. Like estrogen, progesterone can advance the day of estrus but treatment must be given on the third, rather than the second, day of a five-day cycle (84). Furthermore, progesterone has been reported to elevate plasma LH levels (38, 215). Thus, progesterone and estrogen both have biphasic influences over gonadotrophin secretion.

4. Pituitary and Plasma LH and FSH during the Estrous Cycle

Early studies on gonadotrophin secretion revealed that during the afternoon of proestrus an ovulatory discharge of hormones occurred between 2:00-4:00 PM; removal of the pituitary from a population of rats before 2:00 PM resulted in total suppression of ovulation, whereas removal after 4:00PM did not interrupt ovulation (87) (further discussion of this phenomena is in section I.D.2.). Since the development of assays specific for the gonadotrophins (224, 261), as outlined below, various investigators have obtained more direct evidence for gonadotrophin secretion during the afternoon of proestrus and throughout the remainder of the cycle.

a. LH

The first characterization of the dynamic changes occurring in both pituitary and plasma LH was presented by Schwartz and her colleagues (207, 249); it was shown that pituitary LH content is relatively low at metestrus, somewhat higher at diestrus, and reaches maximal levels by the morning of proestrus. LH content begins to drop during the afternoon of proestrus and is significantly lower by the morning of estrus. These findings have been confirmed by other laboratories (8, 166). The proestrus-to-estrus drop in pituitary LH content apparently reflects a surge of LH release during the afternoon of proestrus. Plasma LH levels rise abruptly from marginally- or non-detectable levels of LH at 10:00 AM to readily detectable levels by 3:00PM (8, 166, 176, 236, 250). Plasma LH levels remain high until 8:00PM (176), although it appears only essential that they remain high until 4:00 PM since hypophysectomy after 4:00 PM does not interrupt ovulation (87). This prolonged LH release beyond 4:00 PM may be a physiologic over stimulation "guaranteeing" ovulation, or it may be essential for stimulating the secretion of sufficient progestins for induction of estrous mating behavior (176).

The development of a radioimmunoassay for rat LH (211) with markedly

increased sensitivity has enabled serial measurements from individual rats (212). Using this method (212) bioassay findings for both pituitary and plasma LH have been confirmed. Measurements of serial plasma LH in individual rats during the afternoon of proestrus reveals, as one would expect, considerable variability in 1) onset of the plasma LH rise, 2) maximum amplitude of the rise, and 3) duration of release of the LH surge. This emphasizes the fact that the data from the bioassay speaks only of a population average or trend (212).

b. FSH

Using the FSH bioassay technique of Steelman and Pohley reported in 1953 (261), changes in pituitary and plasma FSH levels have also been delineated. Pituitary FSH content has been found to drop from high levels on the morning of proestrus to low levels on the afternoon of proestrus (119, 202); the drop seems to correspond in time with the drop in pituitary LH. Coincident with the significant drop in pituitary FSH is a significant rise of plasma FSH during the afternoon of proestrus (202). A difference between four- and five-day cyclic rats seems to exist; the drop in pituitary FSH is not as marked in the rats with four-day cycles as in those with five-day cycles (119, 202).

D. Hypothalamic Regulation of the Pituitary-Ovarian Axis

1. Functional Dependence of Pituitary on Hypothalamus

a. Nature of the hypothalamic-pituitary link

While the pituitary's importance to ovarian function was gaining recognition, evidence for an important role of the hypothalamus was also accumulating. Among the first indications of brain involvement in the secretion of the pituitary gonadotrophins were the experiments of Camus and Roussy (42) and of Baily and Bremer (14). Although their primary concern was studying the etiology of diabetes insipidus, they reported, as incidental, atrophy of the reproductive organs after destruction of hypothalamic tissue. Environmental

influences over pituitary-gonadal function were soon demonstrated (34, 96, 102), indicating that the pituitary-gonadal system was dependent on sensory inputs. Thus, ovulation in the rabbit was recognized as being induced by coitus with the pituitary playing a primary role in the process; hypophysectomy stopped ovulation from occurring only if performed within one hour of coitus (96). The environmental light-dark cycle was recognized as an important factor regulating pituitary-ovarian function (34, 102, 143). Constant light induced sporadic cyclicity and, commonly, a state of persistent estrus, i.e., continuous vaginal cornification (34, 102). Changing the lighting schedule by 12 hours put the animals in a cycle 90° out-of-phase with their control state (34). Constant light also induced precocious puberty, while constant dark was found to retard puberty (102). Changing the light-dark cycle was also found to shift the time of onset of vaginal cornification and behavior during the cycle (143).

The possibility that the hypothalamus might be involved in the transmission of information from the external environment prompted investigations of the hypothalamic involvement with the pituitary-gonadal system. Electrical stimulation of discrete regions of the hypothalamus was found to induce ovulation in rabbits (133, 135, 140, 194), whereas lesions of the hypothalamus induced anestrus with atrophy of ovaries and uterus (65, and section I.D.3.), or persistent vaginal estrus with the concomitant markedly follicular ovaries (65) in spontaneous ovulators. Recognizing neural involvement in the control of pituitary secretion, investigators sought the nature of the regulation. Two possibilities presented themselves: 1) a prominent neural link to the pituitary, the hypothalamic-hypophyseal tract, was known to exist (101), and 2) a vascular portal system linking the pituitary to the hypothalamus had been described (231, 276). Either or both anatomical links could serve as communication channels to the pituitary. The first possibility, a direct nerve pathway to the

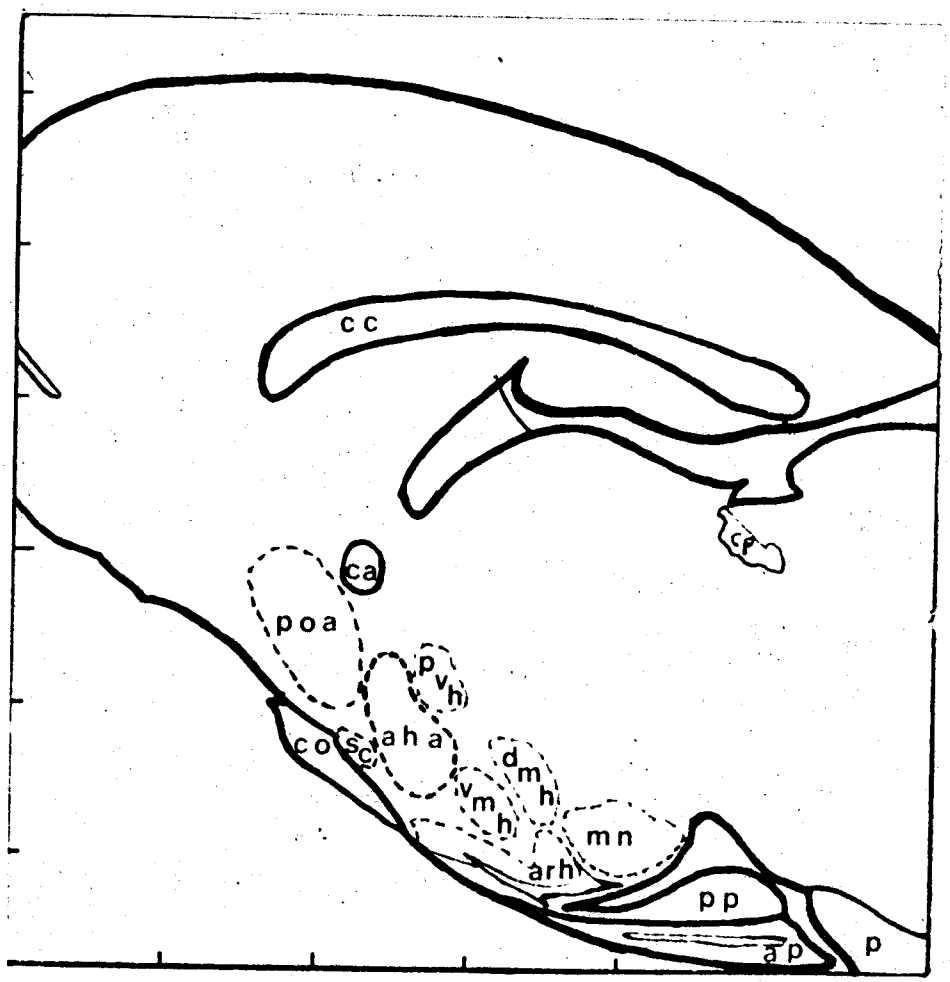


Figure 1.
Anatomical relationship of hypothalamic nuclei and the preoptic area.

ac - anterior commissure
aha - anterior hypothalamic area
ap - anterior pituitary
arh - arcuate nucleus
cc - corpus callosum
co - optic chiasma
cp - posterior commissure
dmh - dorsomedial hypothalamic nucleus
mn - mammillary nuclei
p - pons
poa - preoptic area
pp - posterior pituitary
pvh - periventricular nucleus of the hypothalamus
sc - suprachiasmatic nucleus
vmh - ventromedial hypothalamic nucleus

Modified from De Groot (60).

pituitary, was soon ruled out be various anatomical and physiological studies. Anatomical studies revealed only a very sparse distribution of nerves in the pars distalis and these seemed associated with vascular smooth muscle (122, 239). This contrasted with the abundant nerve supply observed to be passing into the posterior lobe of the pituitary via the hypothalamic-hypophyseal tract (101). Examination of avian and cetacean pituitaries (in which there is a distinct separation of the pars distalis from the neural lobe) failed to reveal any nerve fibers in the pars distalis (70, 134). In addition, following transection of the hypophyseal stalk, atrophy of the neural lobe resulted; the pars distalis was generally little if at all affected (136), although a few labs noted a structural and functional impairment of this lobe (33, 177, 240). Furthermore, stimulation of the hypothalamus was found to induce ovulation while stimulation of the pituitary was unsuccessful (135, 194), thus emphasizing the fact that while the hypothalamus was functionally linked to the anterior lobe of the pituitary, neural connections to or within the adenohypophysis were apparently not involved.

However, despite the lack of evidence for any neural link between the CNS and the adenohypophysis, acceptance of the vascular portal system as the informational link was hampered because the direction of portal blood flow was disputed. Blood was first believed to pass from the pituitary to the hypothalamus (231), but in a later study the possibility of the opposite direction of blood flow was proposed (276). Both of these opinions were based on anatomical evidence; the second, however, was later supported by the direct observation of flow passing into the pars distalis from the hypothalamus in amphibia (121). Evidence for the hypophyseal-petal flow in mammalia was accidentally gained by Green and Harris (122). Some of their preparations intended for examination of the portal system after systemic infusion of India ink had only been partially

infiltrated by the ink. In such "inadequate" preparations, the hypothalamic side of the portal system had been infiltrated while the hypophyseal side had no ink. Direct observation of the portal system in the rat finally revealed the flow was indeed toward the pars distalis (123).

Another impediment to the acceptance of the portal system was the non-uniform results of stalk sectioning alluded to above. Some reports claimed degeneration of the pituitary with resultant ovarian and uterine atrophy (33, 177, 240), while other reports claimed good maintenance of reproductive function (62, 63). Building on a previous demonstration (137) that the portal vessels, unlike the nerves in the hypophyseal-hypothalamic tract, have great regenerative ability, Harris (138) demonstrated the importance of revascularization to the functional state of the pituitary following stalk sectioning. A small piece of waxed paper was placed between the hypothalamus and pituitary in some animals after stalk transection. Only when portal vessels managed to regenerate around the edges of the waxed paper was there any progress toward a regaining of pituitary function.

A second approach which demonstrated that the functional integrity of the pars distalis indeed depended on its vascular connections with the hypothalamus was that of hypophyseal transplants (125, 139, 219, 229). It had initially been demonstrated that homotransplants of pituitaries in the sella turcica function normally (125), whereas the cessation of estrous cyclicity after hypophysectomy was not overcome by intramuscular implants of pituitaries (229). Harris and Jacobsohn (139) extended these observations by hypophysectomizing postpartum rats and, immediately thereafter, transplanting pituitaries from their young under either the median eminence or the temporal lobe. Those animals receiving transplants under the median eminence resumed full reproductive capacity, i.e., vaginal cyclicity, normal ovaries and uteri, pregnancy,

and lactation. The only abnormality was the lack of the milk let-down reflex, which could be explained by the lack of regeneration of the neural lobe since injections of oxytocin corrected the disturbance. In contrast, rats receiving transplants under the temporal lobe did not resume normal reproductive function, despite the fact that revascularization of the pituitaries in both sites was rich. The concept of a neurohumoral link by the transplant method was elegantly finalized when Nikitovitch-Winer and Everett (219) demonstrated that pituitaries completely removed from the sella turcica and transplanted onto the kidney for one month would not support reproductive functions; however, they would reinstate reproductive phenomena if retransplanted under the median eminence but not if retransplanted under the temporal lobe. These experiments provided strong evidence for the presence of some critical substance(s) in the portal blood, but not (at least in as great a quantity) in either the general systemic vascular system or in blood from "nonspecific" nerve tissue.

b. Endocrine nature of the hypothalamus

If the informational link between the hypothalamus and the pars distalis was indeed vascular, both the hypothalamus and plasma should contain substances which stimulate pituitary secretion. The first demonstration of a hypothalamic extract which could elevate gonadotrophin secretion was presented by McCann and co-workers (201). Other laboratories (40, 41, 218) also reported an extract of the hypothalamus which, like McCann's (200, 201), induced LH secretion. The active component(s) of the extract did not appear to be neurohypophyseal hormones (40, 41, 201), serotonin (40, 41, 218), histamine (40, 41, 218), or epinephrine (40, 41, 218). Using highly purified preparations of LH-releasing factor(s) (LH-RF), Schally and Bowers (243) subsequently demonstrated the ability of LH-RF to stimulate LH release in vitro as well as in vivo.

Early characterization of the LH-releasing substance(s) was presented

by McCann (195). Ten minutes of boiling destroyed the activity of LH in pituitary extracts but not of the LH-releasing activity in stalk median eminence (SME) extracts. However, LH-releasing activity of the SME extracts was found totally inactivated by incubation with pepsin, thus leading to the belief that the LH-RF was a polypeptide. These initial observations were supplemented later when highly purified LH-RF was prepared and analyzed by several laboratories (95, 244). Although Fawcett et al. (94) prepared highly purified extracts capable of inducing ovulation, they were unable to characterize the structure because of insufficient material; however, they were able to distinguish two separate biologically active fractions. Schally and Bowers characterized the amino acid content of the LH-RF they separated (244), although no details on structure were presented.

Carotid artery injections of this latter highly pure LH-RF (244) were shown to have a dose-response effect on inducing ovulation (11); LH contamination was ruled out by the fact that ovulation was not induced in hypophysectomized rats by the LH-RF. A marked increase in plasma LH has been noted within ten minutes of an injection of SME extract (117).

Knowing that plasma and pituitary LH fluctuates during the estrous cycle, one may expect the LH-RF to also fluctuate. With this in mind, hypothalami were collected from rats during the estrous cycle (44, 237) and their LH-releasing activity estimated. Although two laboratories noted a cyclic change in potency, their results differed. One (237) demonstrated high potency during metestrus, diestrus, and proestrus AM with a sudden drop during proestrus PM. Levels remained low through the morning of estrus, but high levels were again observed by the afternoon of estrus. The other laboratory (44) found constant low levels throughout the cycle with a single peak during late diestrus and a quick return by the morning of proestrus to control levels.

This difference may be related to the difference in strains used and/or to the differences in the environmental lighting schedule of the animals' rooms.

Evaluation of ovarian steroid feedback effects reveals no elevation of LH-RF in hypothalami of ovariectomized rats (44); in fact, a drop of LH-RF has been reported (230), which may indicate that, unlike in the pituitary, storage and secretion within the hypothalamus are closely linked.

If the hypothalamus is secreting factors affecting the pars distalis, these factors should be detectable in the blood as well as in hypothalamic extracts. Nallar and McCann (216) reported LH-RF-like activity in the systemic blood of rats ovariectomized for two to three months; much less activity was found with lesions destroying the median eminence. Activity was not detectable in cyclic rats nor in rats ovariectomized for one week, indicating a prolonged absence of negative feedback effects was needed to obtain detectable plasma levels of LH-RF. These results explain the loss of pituitary function when transplanted to the kidney or temporal cortex (section I.D.1.a.). With the development of a technique for collecting the hypothalamic portal effluent (232), the important comparison of LH-RF activity in portal blood versus systemic blood could be made. Detection of LH-RF in portal blood plasma was achieved by *in vitro* incubation of pituitaries with collected plasma. Assay of the incubation medium revealed that significant LH had been released after addition of portal blood plasma, but not after addition of femoral vein blood plasma (153).

Since high activity has been found in extracts of SME, ventral hypothalamus immediately over the SME, and the "chiasmatic" region (including the optic chiasma, supraoptic nucleus, suprachiasmatic nucleus, and some of the preoptic area) (195), it appears that the neurosecretory neurons may originate as far anterior as the preoptic area. Recent reports are beginning to link

LH-RF activity with what may be the neurosecretory granules isolated from the SME region (151).

FSH-releasing factor(s) (FSH-RF) activity was first reported in extracts of the SME in 1964 (149). While SME extracts elevated plasma FSH in estrogen-progesterone treated ovariectomized rats, cerebral cortex extracts did not. This property was lost when the pituitary was removed, ruling out FSH contamination. The SME extracts were still active after median eminence lesions (see section I.D.3.) had dropped the plasma FSH levels in ovariectomized rats. Initial attempts to separate the FSH-RF activity from LH-RF activity were unsuccessful (150); this study did, however, demonstrate a separation of LH-RF and vasopressin activity. Further work separated FSH-RF and LH-RF activity (67). FSH-RF activity from ovine and bovine sources has been reported in both in vitro and in vivo experiments (152, 170). Although there is current discussion over whether the releasing factors are really polypeptides or not (245), the recent discovery of the structure of thyroid stimulating hormone-releasing factor and its synthesis (31, 36) may serve as a model for the other hypothalamic factors, i.e., small molecules with peptide linkages (198).

c. The hypophyseotrophic area of the hypothalamus

The strong indications of a functional dependence of the pituitary hypothalamic humors (section I.D.1.a. and b.) encouraged studies on the histological and functional maintenance of intrahypothalamic pituitary implants (103, 131, 132, 165). The 1962 studies revealed a specific hypothalamic region in both males (165) and females (131) which is capable of supporting pituitary histology. This hypophyseotrophic area (HTA) (131, 265) was found to extend from the preoptic area and anterior hypothalamus to its posterior most border ventral and anterior of the premammillary body nuclei. The paraventricular nucleus forms the anterior dorsal boundary, and the area extends

ventrally encompassing the ventromedial and arcuate nuclei (Figure 1). The lateral extent is not not much more than one millimeter. The HTA contains active hypophyseotrophic substances, as indicated by the fact that in pituitary grafts straddling the HTA only that portion of tissue adjacent to or within the HTA is maintained (265); juxtaposition of the pituitary graft with the capillary loops of the median eminence is not essential for histologic maintenance (131). Physiologically active releasing factors have been isolated from within the rough boundaries of the HTA (265).

Extension of these findings to functional maintenance of implanted pituitaries was presented in 1965 (103, 132). Ovarian weights above hypophysectomized levels, and resumption of vaginal cyclicity, were only found when pituitary implants were in the HTA (103, 132). Although mating was reported, pregnancy did not result (103); furthermore, Flament-Durand's examination of the ovaries revealed many follicles of various sizes but no corpora lutea, indicating insufficient release of LH for either ovulation or luteinization. Halász et al. (132), however, did find corpora lutea along with developed follicles but mating and ovulation were not directly evaluated by Halász.

Since both laboratories described the same HTA and both found resumption of vaginal cyclicity, no obvious explanation presents itself for these conflicting reports of corpora luteal development. Neither gave details on the specific location of the implants supporting vaginal cyclicity and ovarian weights; thus, it may be that the two laboratories placed the active implants in regions of differing sensitivities to the feedback effects (see section I.D.5.) of pituitary hormones. Thus, the local LH levels may have affected LH-RF secretion differently with different implant locations. A second possibility is that placement of the implants in the former study (103) may have cut or destroyed vital afferent nerve tracts to the hypothalamus responsible

for initiating the release of an ovulatory surge of LH (see section I.D.3.). Nevertheless, the results of the above studies demonstrate that pituitaries implanted within specific regions of the hypothalamus can support ovarian function.

2. Circadian Stimulation for Spontaneous Ovulation

The physiological stimulus for spontaneous ovulation in the rat was first shown to have a circadian quality by Everett et al. (92, 93). Ovulation was blocked completely when drugs such as atropine, dibenamine, and barbiturates were administered at proestrus before 2:00 PM, but only partially when treatment was given between 2:00 PM and 4:00 PM; no blockade occurred if treatment was given after 4:00 PM (92, 93, 94). When allowed to recover from the drug, the blocked rat ovulated one whole day later - not after the drug wore off; this ovulation can also be blocked by a second injection given before the critical period (2:00 - 4:00 PM), with ovulation being retarded an additional full day. This process of properly timed injections can be followed until the ripe follicles atrophy. Further evidence for a circadian ovulatory stimulus is that properly timed estrogen and/or progesterone injections advance or delay the time of LH release and ovulation, again by 24 hours (86, 92). Postpartum ovulation also reflects the circadian nature of the ovulatory stimulus. If parturition occurs before the critical period, ovulation occurs that night; however, if parturition occurs after the critical period, ovulation takes place the following night (146).

Associated with the critical period during proestrus is a rapid elevation of plasma LH and a drop in pituitary LH; such changes are absent if the critical period is blocked (248, 249, 250). A significant rapid elevation of plasma LH also occurs in chronically ovariectomized female rats during what would be the critical period of the intact proestrous rat (176), indicating that this circadian periodicity in CNS-pituitary activity is independent of

hormones of ovarian origin. The demonstration of the critical period in immature female rats induced to ovulate with exogenous hormones (203, 205), implies that the neural mechanism essential for ovulation is developed at an early age; however, McCormack and Meyer have noted that the minimal age at which ovulation can be induced is 18 days of age (204).

This circadian stimulus seems unique to the female since ovaries transplanted into males do not cycle, but develop cystic polyfollicular ovaries (189). In addition, we have failed to detect a 24 hour periodicity in pituitary or plasma LH in adult intact or castrated males (176). Since male pituitaries transplanted into females will support ovulation (228), the lack of an ovulatory impetus must be centered in the CNS rather than the pituitary. Testicular transplants (228) or androgen injections (15, 253) in female rats during their first week of life, however, induce a persistent-estrous state characterized by continuous vaginal cornification, failure to ovulate, and polycystic ovaries similar to those transplanted into males (183, 189). Thus, neonatal exposure to androgen appears to abolish the cyclic quality inherent in the female central nervous system (120).

3. Hypothalamic Ovulatory Stimulus

Dey and co-workers (64, 65, 66), using guinea-pigs, first described two reproductive dysfunctions following hypothalamic lesioning and their association with separate regions of the hypothalamus. An anestrous state was found to be associated with lesions in the basal hypothalamus reflecting total or near total shutdown of pituitary gonadotrophin secretion. In contrast, lesions in the anterior hypothalamic-preoptic region induced a persistent-estrous state. In this second dysfunction the follicular ovaries indicated that FSH was being secreted; secretion of LH was felt to be abnormally low since luteinization did not occur, but apparently was not absent, since the

interstitial tissue and estrogen levels appeared well maintained (64, 65). The implications of these data were only realized through other evidence from varied sources as discussed below. Reference to Figure 1 may be helpful while reading this section.

a. The basal hypothalamus

Localization of lesions in the arcuate nucleus and median eminence resulted in gonadal atrophy in both males (27, 28) and females (56, 108, 110, 197). As measured by a total gonadotrophin bioassay, loss of pituitary gonadotrophin potency follows lesioning in the region of the arcuate nucleus (27, 28). Specific assays for LH, following lesioning of the median eminence region, confirmed the previous results; a marked drop in pituitary LH potency was noted, which could not be elevated by bilateral ovariectomy as it could in the non-lesioned animal (267). Realizing that the arcuate nucleus-median eminence region is the posterior extent of the HTA (131), it is not surprising that destruction of the delivery point of the releasing factors from the hypothalamus to the portal system leads to loss of pituitary function. The arcuate nucleus apparently is more important for reproductive cyclicity than the median eminence, since vaginal cyclicity continues after surgical removal of the median eminence, but ceases if the arcuate nucleus or parts of it are removed along with the median eminence (164).

As discussed earlier (section I.D.1.a.), stimulation of the basal hypothalamus, but not of the pituitary, induces ovulation in rabbits (135, 194). Similarly, rats in which spontaneous ovulation has been blocked by pentobarbital can be induced to ovulate by stimulation of the basal hypothalamus from the arcuate nucleus to the optic chiasma (55) - the approximate ventral boundaries of the HTA (131). Isolation of the basal hypothalamus by an ingenious knife-cut technique (130) has revealed that this region is capable of maintaining

some pituitary functions, but is not able to support ovulation without neural connections from the anterior hypothalamus (129). Thus, cutting just the anterior connections leads to an anovulatory persistent-estrous state; cutting all posterior and lateral connections, however, is compatible with ovulation (129). Thus, the HTA must have specific anterior connections for the cyclic release of gonadotrophins.

b. The anterior hypothalamus

The persistent-estrous state first observed by Dey after anterior hypothalamic lesions in guinea-pigs (64, 65) also occurs in rats (20, 127, 145, 273). Small localized lesions destroying only the suprachiasmatic nucleus (SchN) will cause persistent estrus (20); ovulation can be induced in these animals by progesterone (20), which presumably acts via other anterior hypothalamic sites since animals with more extensive lesions involving the POA do not ovulate after progesterone treatment (20, 127, 273). Stimulation of the basal hypothalamus in the latter animals activates the pituitary to induce ovulation (20), further indicating the HTA and pituitary require some afferent inputs for LH secretion to exceed tonic levels.

As mentioned earlier (I.D.2.), transplanted testes (228) or androgen injections (15, 253) can also induce persistent estrus; the action of the androgen is not on the pituitary, though, since male pituitaries support female cyclicity (139). As in animals with POA lesions (20, 127, 273), progesterone injections in the androgenized females fail to induce ovulation whereas stimulation of the basal hypothalamus induces ovulation (18). Stimulation of the POA in these animals, however, did not lead to ovulation (18) despite its success in inducing ovulation in the barbiturate-blocked proestrous rat (91). It was hypothesized from these data that androgens, like lesioning, removes the capacity for the activation of the POA by environmental lighting and hormonal

feedbacks stimulating the basal hypothalamus and resulting in ovulation (18).

Out of these studies grew a formal theory of the dual nature of hypothalamic control over gonadotrophin secretion (18, 120). The basal hypothalamus (HTA) was termed the first level, regulating the secretion of the tonic or basal gonadotrophins necessary for follicular development and steady-state estrogen secretion; this area is incapable, however, of initiating the ovulatory discharge of pituitary gonadotrophins. The second level, the POA-SchN area, was seen as periodically stimulating the first level, thereby inducing ovulation. While the first level depends on activation by the second level for its release of an ovulatory surge of gonadotrophins, the second level requires a combination of favorable events, exogenous (environmental) as well as endogenous (hormone feedback), for its activation.

This theory is well supported by recent experiments outlined below. Structural (69, 210) and metabolic (69) differences have been described for the anterior hypothalamus of males and females. In support of the concept of sequential activation, localized intrahypothalamic injections of pentobarbital revealed two "critical periods". Injections into the POA must be made before 4:00 PM to block ovulation, while injections into the basal hypothalamus can be done as late as 8:00 PM. This implies a sequential activation beginning within the POA, then passing to the basal hypothalamus and requiring several hours for full activation (128). Direct evidence for the transfer of activation was provided by the demonstration that barbital-blocked proestrous rats stimulated in the POA show elevations of multi-unit activity within the median eminence-arcuate nucleus region only if ovulation occurs (269).

Further convincing evidence is provided by Halász' hypothalamic knife-cut technique (130), as noted previously. Either total or anterior isolation of the HTA is not compatible with ovulation, while posterior and lateral isola-

isolations are compatible with ovulation (129, 130). Most recently (168), isolation of the POA-HTA as a unit, difficult because of the high mortality rate, has been achieved and shown to be compatible with vaginal cyclicity, ovulation, and corpora lutea formation. The POA is therefore capable of supporting spontaneous ovulation; however, since vaginal cyclicity was not regular, extrahypothalamic influences are probably essential for maintaining the coordination of physiologic phenomena necessary for normal reproductive cyclicity.

4. Ovarian Hormone Feedback

Ovarian steroids are capable of exerting either facilitatory or inhibitory actions on gonadotrophin hormone secretion depending upon the preinjection hormonal state of the animal (section I.C.3.). As discussed below, these biphasic steroidal actions are apparently the result of an interaction of progesterone and estrogen with both the CNS and adenohypophysis.

The possibility of a direct effect of ovarian steroids on hypothalamic structures was first studied by implanting bits of ovarian tissue (109) and crystalline steroid (59, 155, 184, 256) into the brain. Gonadal atrophy (59, 155, 184), inhibition of the postcastration rise in pituitary and plasma LH (156, 233), and the inhibition of castration cells (154, 185) following median eminence implants of estrogen have been reported, suggesting that this region mediates a negative feedback effect of estrogen. This was disputed, however, by Bogdanove (29) who found no effect on pituitary castration cells if implants were in the median eminence, but localized inhibition of castration cells in the immediate vicinity of intra-pituitary implants. Bogdanove (29) felt the reason others found negative feedback only from the median eminence implants and not from the pituitary implants was that the estrogen was well distributed from the hypothalamus by the portal system to the pituitary, thus directly affecting the pituitary; with intra-pituitary implants, on

the other hand, the distribution of estrogen throughout the gland was poor. This possibility was supported by the findings of Ramirez et al. (233), who reported lower plasma LH but not pituitary LH with adenohypophyseal implants of estrogen. In addition, studies with tritiated estrogen implants in the median eminence resulted in significant levels of radioactivity throughout the pituitary gland, while with similar implants in the pituitary the spread of radioactivity tended to be localized near the implant (222). This same study, however, showed that while pituitary implants led to hypophyseal hypertrophy (i.e., a direct effect), pituitary LH levels were not affected. Median eminence implants, while causing little hypophyseal hypertrophy, did lower pituitary LH, thus indicating that estrogen had no direct effects on storage of LH, but had powerful indirect effects via the hypothalamus. Estrogen implants in the POA also appear to inhibit ovarian compensatory hypertrophy (97, 186), thus complementing the lesion studies indicating that the POA is essential to feedback phenomena (details below). The POA seems capable of mediating a facilitative influence also, in that estrogen implants will accelerate the onset of puberty (255). Hypothalamic implants of progesterone also reveal a negative feedback effect at the median eminence, but not at the POA (256). The interpretation of this study must be made carefully, however, in the light of Bogdanove's criticism of estrogen implants (29) and since progesterone is suspected of interfering with the pituitary responsiveness to the hypothalamic releasing factors (262).

Direct feedback influences of the ovarian steroids on the CNS are strongly supported by autoradiography studies indicating radioactivity within nerve cells in localized regions of the CNS (7, 264). Uptake of tritiated estradiol has been reported in the arcuate and POA nuclear regions of the hypothalamus (7, 264) as well as the posterior third of the amygdala (7) but not

elsewhere in the CNS (7, 264). High quantities of radioactivity are also found in pituitary tissue following systemic injections of tritiated estradiol, thus indicating that the pituitary may also be a direct feedback site (124, 158). Although estrogen binding to hypothalamic structures has recently been shown to vary with the stages of the estrous cycle, this may be a reflection of the changing endogenous estrogen levels during the cycle (157). Estrogen has also been shown capable of elevating peptidase activity in the hypothalamus (100) and of inhibiting C^{14}-lysine incorporation into both the amino acid pool of the hypothalamus and the proteins of nuclear regions of the basal hypothalamus (187). Thus, the gonadal steroids can cross the blood-brain barrier (277) for intimate functional contact with CNS structures.

That ovarian steroids can modify CNS functioning has been demonstrated more emphatically by reports of electrophysiological changes in the CNS following progesterone and estrogen injections (90, 266). In estrogen-primed rabbits, electroencephalographic (EEG) arousal thresholds have been reported to be initially elevated by progesterone (0.5 mg) but markedly lowered by the next day (161). Cortical EEG patterns in female rats have also been reported depressed following subanesthetic doses of progesterone (0.3 mg) (10). Studies utilizing animals with chronically implanted electrodes reveal a characteristic elevation of regional multi-unit activity of the basal hypothalamus and POA during the critical period (163). Significantly, progesterone-induced ovulation advancement was accompanied by an advancement of the multi-unit activity to the new critical period. Elicitation of single unit activity by peripheral stimuli has been found to vary with the stages of the estrous cycle (17). Changes in single-unit activity after progesterone (17) and estrogen (180, 182) have been reported. Interpretation of these electrophysiological results must be done carefully in that some carrier vehicles (e.g., propylene glycol and

ethanol) have been shown to alter EEG and single-unit activity (181).

The sensitivity of neural structures to stimulation-induced ovulation seems to shift during the estrous cycle; the earlier in the cycle one stimulates the POA, the more difficult it is to induce ovulation. However, the variability could as likely stem from any point(s) distal to the POA stimulation site (147).

Electrolytic lesioning of the CNS has provided an invaluable technique for defining CNS functions in maintaining the estrous cycle. As discussed earlier (section I.D.1. and I.D.3.), POA lesions result in follicular ovaries which do not rupture, and continuous estrogen secretion (64, 65, 127, 145). The maintained estrogen levels, however, are inconsistent with data indicating that estrogen s hould decrease plasma FSH and ultimately, estrogen levels (37, 113, 144, 266). Thus, it seems that the POA contains elements directly and/or indirectly involved in estrogen feedback regulation of FSH (266), as well as the secretion of LH and the ovulatory discharge of gonadotrophins (64, 65, 127, 145, 273). This is supported by the fact that in intact immature rats parabiotically united with ovariectomized immature rats, large follicular ovaries develop and uterine weight increases (104); treatment of the ovariectomized partner with estrodiol prevents such changes, unless the rat also has a POA lesion. In addition, POA-lesioned rats treated with low physiological doses of estrogen maintain histologically normal ovaries but non-lesioned rats when treated with the low estrogen doses develop histologically atrophic ovaries, thus indicating a deficiency in estrogen feedback suppression of FSH (105). Some less sensitive feedback route exists, however, since very high doses of estrogen (25-50 μg) will inhibit FSH secretion in intact and POA-lesioned rats as evidenced by ovarian histology (266). Furthermore, POA lesions remove the normal reciprocal estrogen-FSH relationship as evaluated by ovarian compensatory

hypertrophy, i.e., ovarian compensatory hypertrophy does not occur in POA-lesioned rats (57, 106). The evidence is thus strong that the POA and/or some structure(s) conducting information into the POA, is indispensable for the feedback regulation of FSH. The evidence also implies that the basal hypothalamic-pituitary unit does not sense estrogen changes for FSH regulation.

The effects of POA lesions on pituitary LH content appear to depend upon the size of the lesion (32, 267). Large lesions are found to reduce LH content (267) but smaller lesions do not reduce LH content (32). Even though pituitary LH content is reduced by the more extensive POA lesions, it increases following bilateral ovariectomy as does the plasma LH (267). Therefore, while the POA may play a significant role in the ovulatory discharge of LH (64, 65, 127, 145) and the maintenance of proestrous levels of pituitary LH (267), the units distal to the POA seem capable of sensing the loss of ovarian steroids. Evidence of augmented LH secretion in POA-lesioned rats is also shown by the fact that removal of one and a half ovaries results in luteinization of the remaining half ovary (107). Thus, while the basal hypothalamus-pituitary unit appears capable of mediating a negative feedback regulation of LH secretion, the POA is apparently necessary for full regulation. As discussed earlier, although the POA may be essential for maintaining the ovulatory surge of LH, apparently extrahypothalamic influences are needed to maintain the steady four- or five-day cyclic pattern of the estrous cycle (168).

Further evaluations of estrogen feedback by measuring pituitary and plasma LH after injecting various physiological doses of estrogen into ovariectomized normal and neonatally androgenized females imply negative feedback effects on the hypothalamus, but a positive feedback effect on the pituitary (19). Since there is evidence that estrogen does not interfere with the action of releasing factors (235), it may have a direct effect on pituitary secretion;

this is supported by a recent elegant in vitro study (247).

The facilitation of ovulation by progesterone (section I.C.4.) seems to involve specific anterior hypothalamic structures. A single injection of progesterone can induce ovulation following placement of very localized lesions of the suprachiasmatic nucleus, which resulted in persistent estrus, but is ineffective if the lesion is more extensive, involving part of the medial POA (MPOA) (16, 20) or more extensive lesions of the POA (127). Moreover, lesions of only the MPOA, while not resulting in persistent estrus, do prevent ovulation advancement in these cyclic rats (16, 20).

5. Gonadotrophin Feedback

Gonadotrophins also appear to have feedback effects on both brain electrical activity (159, 161, 270) and pituitary secretions (9, 46, 58, 221). The rabbit EEG arousal threshold is elevated following FSH injections while the threshold is lowered by LH (161). The activity of units monitored in the cat hypothalamus seems to be affected by LH; i.e., some units show increased or decreased activity following LH injections (159). Rat hypothalamic multi-unit activity (MUA) has also been reported altered by LH (270); estrogen-treated ovariectomized rats showed elevated MUA in the arcuate nuclear region while simultaneously showing a depression of activity in the POA following LH injections. Since the rise in arcuate nucleus activity was not seen after isolation of the basal hypothalamus, but POA activity still dropped, LH detection may occur outside of the basal hypothalamus. The overall effect of an LH feedback, therefore, seems to be positive if one assumes the elevated activity of the basal hypothalamus indicates LH-RF secretion. Evidence derived from LH or LH-RF measurements, however, strongly indicates that the feedback is negative; hypophysectomy elevates plasma LH-RF from nondetectable levels in ovariectomized rats to detectable levels (216), and LH implants in the median eminence lower

pituitary (46, 58) and plasma LH (58).

Using the ovarian compensatory hypertrophy response (OCH) as an estimate of FSH secretion in neonatally androgenized female rats, Arai and Gorski (9) found that hypothalamic implants of FSH inhibited the OCH and thus presumably FSH secretion, while implants in other parts of the brain or pituitary had no effect. These effects, however, may be related to the androgenization, and thus unique to the male rather than the female (221). The FSH feedback in the normal female has been interpreted as both positive (220, 221) and negative (47, 48, 111). This discrepancy may be related to the age of the experimental animals, i.e., prepubertal (220, 221) or postpubertal (47, 48).

It thus appears that LH and FSH exert negative feedback effects through the CNS in the adult rat. These feedback effects may be exerted via the general systemic circulation and/or a short vascular loop conveying blood from the adenohypophysis back up into the hypothalamus (266).

The further possibility of regulative feedback effects of the releasing factors must also be recognized as possible.

E. Neuroanatomical Connections Between the Hypothalamus and Amygdala

Although anatomical connections between the hypothalamus and the rest of the CNS are rich (239, 266), this section will only consider the hypothalamus' informational link with the amygdala.

Using axon degeneration techniques, which were later called into question (52), the stria terminalis (ST) was shown to convey fibers from the amygdala to the dorsomedial and ventromedial hypothalamic nuclei of the monkey (1). Using better techniques, the connection between the amygdala and the hypothalamus was confirmed, but a different distribution of ST fibers was established (217). Fibers from only the posterior amygdala (the corticomedial nuclear group) were found to project through the ST to the medial preoptic

area (MPOA) and medial anterior hypothalamus. No projections to the ventromedial nuclear region were found. Some ventral degeneration of fibers to the lateral hypothalamus was described, however. These results in the monkey were also noted in the cat (266) and rat (52). In that the POA has been demonstrated to require extrahypothalamic connections to maintain the periodicity of the estrous cycle (168), fibers from the amygdala terminating in the POA may be involved in maintaining reproductive cyclicity.

A large diffuse tract of fibers has also been reported to pass horizontally from the amygdala into the hypothalamus (169, 266); however, since a large diffuse tract of fibers persists after destruction of the pyriform cortex surrounding the amygdala (52), interpretation of the horizontal tract following amygdalar lesions is difficult. Lesioning the hypothalamus will also result in degeneration of fibers along both the ST and the horizontal pathway (52); thus, there appear to be afferent and efferent fibers between the hypothalamus and amygdala.

Evoked-potential studies reveal that stimulation of the amygdala can increase or decrease hypothalamic unit activity, depending on the site stimulated (72, 214); the corticomedial portion of the amygdala tends to inhibit hypothalamic activity, whereas the lateral region tends to stimulate hypothalamic activity. This regional functional dissociation is discussed further in section I.F.

F. Regulatory Influence of the Amygdala over Gonadotrophin Secretion

Ovulation following stimulation of the amygdala was reported as early as 1954 (167). Differential responsiveness of the various amygdaloid regions was demonstrated, with the best results obtained by stimulation of the medial region; ovulation was obtained less frequently when the cortical amygdaloid nucleus was stimulated, and stimulation of the lateral and central nuclear

regions was totally ineffective. These findings are supported by work of other laboratories (141, 160). Ovulation and elevated progesterone levels have been noted following stimulation of the medial amygdala (141, 162), while stimulation of the basal or lateral regions of the amygdala failed to induce ovulation and was accompanied by a drop in progesterone levels (141). Ovulation has also been reported in the cat after amygdaloid stimulation (254) and in the constant light-induced persistent-estrous rat (35). Additional evidence for the involvement of the amygdala in the ovulatory process is that an EEG after-reaction similar to that following coitus in the female rabbit can be evoked by amygdaloid stimulation (160).

The amygdala in the constant light-induced persistent-estrous rat (272) seems to differ somewhat from the rabbit (141, 162, 167). Although the medial amygdaloid nucleus is still the most effective stimulation site for inducing ovulation, moderate success can be attained from stimulation of the basal and lateral nuclear regions, and stimulation of the cortical nucleus is apparently ineffective. This should serve as a caution for careful extrapolation of data between species and especially between species with different ovulatory mechanisms, i.e., reflex versus spontaneous ovulation.

Total destruction of the amygdala in the male results in atrophy of the testes (278), implying that the nuclear group as a whole exerts a net stimulatory effect on the release of gonadotrophins. If, however, exposure of males and females to a different hormonal environment neonatally accounts for the functional sex difference of the POA (120), such exposure may also influence the functional capacity of other brain regions, directly or indirectly. Thus, one must be very cautious in extrapolating data from one sex to the other. This caution is justified by a recent report showing different effects of amygdala stimulation in male and female rats (272).

That a specific region of the amygdala can have an inhibitory effect

over gonadotrophin secretions in the rat was demonstrated by a series of experiments by Critchlow and Elwers (22, 76, 77); lesions of the cortical and medial nuclear regions of the amygdala, or of the stria terminalis, advanced the onset of puberty, while stimulation of these sites retarded the onset of puberty. Additional evidence in support of an inhibitory effect from the amygdala has been attained from studies on the deermouse (73, 74); pituitary and plasma LH became elevated following lesions of the basolateral region as do FSH-RF levels in the hypothalamus.

Electrophysiological studies lend support to the notion that the amygdala has two regions functioning in opposition to one another (268). In intact female rats, the seizure threshold of the medial amygdala is lower during proestrus than during the rest of the cycle, while the lateral amygdala has a higher threshold during proestrus than the rest of the cycle.

Reports that the posterior third of the amygdala accumulates radioactivity following systemic adminstration of H^3-estradiol (7), and that estrogen implants in the amygdala induce lactation in the rabbit (271), makes the amygdala a likely estrogen feedback site. This possibility is also supported by estrogen implant studies on ovariectomized rats (175). Since lesions of the cortical nucleus or destruction of the stria terminalis elevated plasma LH, and estrogen implants in the corticomedial nucleus maintained high plasma LH in spite of high plasma estrogen, estrogen may suppress an inhibitory influence by the amygdala on LH secretion, thereby facilitating LH secretion. Further evidence for an ovarian steroid effect on the amygdala is that ovariectomy abolishes the cyclic changes in seizure threshold noted in the medial and lateral amygdala, and a single injection of estrogen reinstates the cyclic changes with these two regions out of phase (268). In addition, oxygen metabolism of the amygdala was found to be accelerated by estrogen (246), thus supporting the studies

indicating estrogen can alter the physiology of the amygdala. Although the amygdaloid neurons may be directly influenced by hormones, it would be difficult to distinguish direct from indirect hormonal influences since the amygdala has both afferent and efferent fibers (see section I.E.).

Even though the amygdala seems to be involved in the secretion of the pituitary gonadotrophins, massive lesions of the amygdala in the rabbit do not interfere with coitus-induced ovulation (241). As discussed previously, however, the amygdala has both facilitatory and inhibitory influences on gonadotrophin secretions; therefore this effect may indicate that the net results of the amygdala's absence can be compensated for by other brain structures. Ablation of specific inhibitory or facilitatory regions of the amygdala may not be so easily overcome.

Few studies have been performed on intact cycling rats to determine a functional role of the amygdala (21, 45). One short report (45) indicates that more cornified vaginal smears per cycle may result following amygdaloid lesions, indicating somewhat prolonged estrogen secretion, and thus higher plasma gonadotrophin levels, thus offering indirect evidence that in the intact cycling rat the amygdala may have a tonic inhibitory influence over gonadotrophin secretion. The other short report indicates that rats developing precocious puberty after corticomedial lesions show normal cyclicity, mating, pregnancy, and rearing of young, whereas lesions of the anterior amygdala do not disturb cyclicity, but do interfere with pregnancy, delivery, and care of young (21). These reports, however, offer no details. In a later study, using the deermouse, Eleftheriou and Zolovick (74) placed lesions in the basolateral region of the amygdaloid complex. The animals were autopsied one, two, or three weeks later and their plasma and pituitary LH levels measured. Elevated plasma LH and lower pituitary LH were found; however, all of

the lesioned animals were pseudopregnant, and no control plasma and pituitary LH values were provided. Thus it is impossible to relate these data to cyclic data.

Chapter II

STATEMENT OF THE PROBLEM

The amygdala is emerging as an important component in the regulation of gonadotrophin secretions. Especially with the recent report of Köves and Halász (168), it is apparent that extrahypothalamic brain regions must play a stabilizing role in the maintenance of the estrous cycle rhythm. Although the involvement of the amygdala is hinted at by a large amount of data (see Chapter I.F.), an understanding of the dynamic role of the amygdala in the spontaneously ovulating rat is hampered since most data are derived from the naturally persistent-estrous rabbit (167) and cat (254), or from the experimentally-induced persistent-estrous rat (35, 272), all of which appear to exist in a more or less static gonadotrophic state. The fact that the amygdala may have two regions functioning in opposition to one another (35, 76, 141, 268) is also a complicating factor.

Since so little was known about the functional importance of the amygdala in maintaining the estrous cycle of the spontaneously ovulating animal, it seemed critical to perform experiments evaluating amygdalar involvement in the maintenance of the estrous cycle. The corticomedial region of the amygdala was chosen for study since its ablation has been demonstrated to affect the maturation of the ovulatory surge system (76). In addition, if its inhibitory influence were preserved into adulthood, as it seemed it might (45, 175), lesioning of the region may induce a persistent-estrous state relatively easily assessed against other types of persistent-estrus (32). Preliminary experiments, however, indicated that lesioned animals showed vaginal cyclicity up to four

months after lesioning. With this information, emphasis was then placed on experiments which would evaluate some of the dynamic internal parameters of the estrous cycle following amygdala lesions to determine whether there were any shifts in the relative timing of events.

Chapter III

METHODS

A. Animals

Sprague-Dawley-derived rats from Simonsen Laboratories, Gilroy, California, were used for all experiments. Received at 60 days of age, the animals were immediately placed in the environmental rat room for a 14-day acclimatization period, after which daily vaginal smears were commenced and continued until autopsy. Lighting was on from 5 AM until 7 PM. Rockland Mouse Breeder Diet (used in place of Rat Chow to minimize variations of natural plant estrogenic contamination in the food) and tap water were available to all rats *ad libitum*.

B. Stages of the Estrous Cycle

The stages of the animals' estrous cycle, as first defined by Long and Evans (1922), were determined from unstained vaginal smears, which were taken by saline lavage, and then examined microscopically. Both the daily smear cytology and the past history of vaginal smear cycles were used to predict the cycle stage on a given day. When necessary, because of an unclear smear or smear history, animals were laparotomized under ether anesthesia during the morning (10-11 AM) and inspected for either uterine ballooning, indicative of proestrus, or for a swollen ampullar segment in the oviduct, indicative of ovulation and therefore estrus. Otherwise, with a clear smear and cyclic history, the animal was inspected just prior to autopsy for a swollen oviduct or uterus.

C. Experimental Groups

Four basic experiments were conducted; throughout the dissertation they shall be referred to as : 1) three-cycle study (3-C), 2) two-month study (2-M), 3) four-month study (4-M), and 4) ovarian compansatory hypertrophy study (OCH). All studies contained the three major groupings of control, sham, and lesioned animals. These groups are further subdivided as explained below. Animals were included in a study on the basis of their pre-surgical cyclicity.

1. Three-Cycle Study

Animals were selected for the three-cycle study only if they demonstrated three consecutive four-day cycles just prior to lesioning and could be used within the first ten cycles. They were sacrificed on or about the third estrus after lesioning or sham lesioning. Control animals were sacrificed in a corresponding age range. Four time periods during the estrous cycle were studied: proestrus 2:30-3:30 PM, estrus 10:00-11:00 AM, estrus 2:30-3:30 PM, and metestrus 2:30-3:30 PM. Bilateral lesions were placed in the cortical amygdaloid nucleus (Table I).

2. Two-Month Study

Animals were selected for the 2-M study only if 1) they demonstrated at least four consecutive four-day cycles immediately preceding surgery, 2) they had no episodes of three consecutive five-day cycles, 3) they had no episodes of pseudopregnancy, and 4) they could be used within the first ten cycles. These criteria were decided upon after the four-month study was almost completed and it became apparent that no obvious alteration in vaginal cyclicity was induced. In case the changes in cyclicity were subtle, the rigid criteria outlined above were adopted in an attempt to obtain a population of rats with functionally homogeneous neuroendocrine networks governing their cyclic pattern. Autopsies were performed as close to 60 days after lesioning or sham lesioning as possible; control animals were autopsied during a cor-

responding age range. The animals were autopsied during three time periods of the estrous cycle: proestrus 2:30-3:30 PM, estrus 10:00-11:00 AM, and estrus 2:30-3:30 PM. Bilateral lesions in this study group were placed in the cortical nucleus or lateral nucleus of the amygdala, or in the dorsal arch of the stria terminalis (see Table 1).

3. Four-Month Study

Animals showing a cyclic pattern of either four- five-day cycles uninterrupted by pseudopregnancy were used in the 4-M study. Autopsies were performed as close to 120 days after lesioning or sham lesioning as possible; control animals were autopsied during a corresponding age range. Two time periods during the estrous cycle were looked at in detail: proestrus 2:30-3:30 PM, and estrus 2:30-3:30 PM. Bilateral lesions were placed in the cortical nucleus of the amygdala. In addition, lesions were placed in regions other than the amygdala (Table 1) to evaluate possible non-specific effects of ions deposited during lesioning, and to evaluate the physiologic significance of the lesion size.

4. Ovarian Compensatory Hypertrophy Study

Animals for the OCH study were selected from among those which were showing four- or five-day vaginal cycles but which did not meet the rigid requirements for inclusion in one of the cyclic studies. Some had undergone a previous period of pseudopregnancy, but had at least three four- or five-day cycles afterwards. These animals were uniterally ovariectomized (left ovary) the same day they were lesioned or sham lesioned. Ten days later they were autopsied; the right ovary was removed and weighed. Lesions were placed in the cortical nucleus of the amygdala, the stria terminalis, or one millimeter below the surface of the cerebral cortex (Table I).

The following chart defines the coordinates for the various bilateral lesions according to de Groot's atlas ((60)).

TABLE I

LESION COORDINATES

Site of Lesion	Anterior	Dorso-Vent.	Lateral
Preoptic-Suprachiasmatic Region (POA)	7.4	-2.1	0.5
Anterior-Lateral Region of Amygdala (ALA)	5.4	-2.5	5.2
Dorso-Medial Nucleus of the Thalamus (DMNT)	5.0	+0.5	1.0
Stria Terminalis (ST)	4.6	+1.7	4.0
Internal Capsule (IC)	4.6	-0.5	4.0
Cortical Region of the Amygdala (ACO)	4.6	-3.5	4.0
Cerebral Cortex	4.6	1 mm below cortex	4.0
Hippocampus (Hipp.)	3.0	-2.3	4.0

D. Lesioning

1. Electrodes

Lesions were placed using unipolar concentric electrodes constructed of stainless steel wire (6 mils, 0.15 millimeters) threaded through 26 gauge stainless steel hypodermic tubing. After three coatings of epoxylite were baked onto one end, a lead was soldered onto the wire protruding from the uncoated end. The junction was reinforced with De Khotinsky cement and a piece of shrink tubing. The protruding wire from the coated end was trimmed so that one millimeter extended out from the hypodermic tubing. After a half-millimeter

of the protruding wire had been scraped free of epoxylite, the electrode insulation was tested using an Ohmmeter by passing the electrode (clipped to one lead of the Ohmmeter through the electrode lead) through a saline film formed in a wire ring clipped to the other lead of the Ohmmeter.

2. Procedures

Only metestrous animals were chosen for lesioning; they were anesthetized with 35 mg/kg of sodium pentobarbital (Nembutal, Abbot Laboratories) intraperitoneally. If the animal seemed light after cutting through the scalp and clearing away the fasciae, 1% xylocaine was applied to the scalp for several minutes before continuing. If the bregma reading was not in accordance with the atlas (60), an attempt was made to reposition the ear bars. The animal was used as a control if this did not correct the bregma reading. After two holes were drilled in the skull, dorsal coordinate readings were taken of the exposed dura and the penetration distance calculated from the instrument zero. If this distance did not correspond to the penetration distance calculated from the atlas, the animal was usually used as a sham. The electrodes were lowered after puncturing the dura with a fine probe. After grounding the animal by placing a saline-coated tapered brass rod into the rectum, one-mAmp d.c. was passed bilaterally for 15 seconds. The brain electrodes were then disconnected from the power supply and removed from the brain; after this the rectal electrode was removed. The skull was blotted free of bone dust and fragments before the skin was sutured. Survival was excellent, i.e., less than 1% mortality.

Sham lesioning involved the same procedures as lesioning, except that no current was passed through the lowered electrodes.

Control animals received no operative procedures on the skull.

E. Measurements

1. Cyclic Studies

a. Indirect evaluations of hormone levels

During the specified time periods, the animals were weighed and sacrificed under ether anesthesia. The thyroid, left adrenal and ovary, uterus, and the anterior pituitary were removed and weighed. The right ovary with attached oviduct was kept for possible later histological sectioning. Dry uterine weights (24 hours at 45-50°C) were also taken. The mean and standard error of the mean were routinely calculated for all organ weights. Significance between two means was accepted if Student's t-test indicated a "p-value" less than .05. If no significant differences were found among the different cycle stages within the control, sham, or lesioned groups, the weights from all cycle stages were combined into one group and the three resulting major experimental groups were tested for significance.

Egg counts were routinely made in estrous animals. The oviduct, isolated from the ovary, was placed under a dissection microscope. Either the swollen ampullar segment was cut free from the rest of the oviduct and the eggs expressed from one end, or the ampulla on the intact oviduct was cut open with a pair of iridectomy scissors and the eggs expressed into the surrounding saline droplet.

Lumenal fluid from the uteri of proestrous animals was weighed by first carefully dissecting out and trimming the uterus free of extraneous tissue. After the uterus was weighed, it was cut open and the fluid drained out, then reweighed. Subtraction of the second weight, the wet uterine weight, from the first weight, gives an estimate of the amount of uterine lumenal fluid. This information, along with a calculation of the per cent dry weight of the uterus (100% X dry weight/wet weight) was used as a qualitative

measure of plasma estrogen levels (13).

b. Direct evaluation of hormone levels

Plasma and pituitary luteinizing hormone (LH) levels were measured using 1 ½- and 4-hour modifications, respectively, of the ovarian ascorbic acid depletion (OAAD) bioassay developed by Parlow (224, 225). The specificity of this assay for LH has been shown by Parlow (224, 225) and McCann (199). Adrenocorticotrophin (ACTH), FSH, and prolactin are either ineffective or are one thousand to ten thousand times less potent in causing OAAD than is LH. FSH and LH do not synergize. Commercial oxytocin (Pitocin) and synthetic oxytocin (Syntocin) are undetectable by the bioassay (199). However, commercial vasopressin (Pitressin), and lysine and arginine vasopressins (synthetic vasopressins), are detectable by OAAD, but only when administered in pharmacological doses. Neither hemorrhage nor injections of Nicotine induce OAAD (199).

Recipient rats for the OAAD bioassay were obtained from ARS/Sprague-Dawley, Madison, Wisconsin at 22 days of age, and were housed in the same room as the experimental donor rats. Rockland Rat and Mouse Chow, and tap water, were available ad libitum. At 26 days of age, these rats received 50 IU Pregnant Mare Serum (Equinex, Ayerst) subcutaneously in 0.5 cc saline; at 28 days of age, they received 25 IU of Human Chorionic Gonadotropin (Antuitrin "S", Parke-Davis) subcutaneously in 0.5 cc saline; at 35 days of age, the rats were used for the bioassay. The format of the bioassay procedure, as described below, depends on whether the test substances are pituitary homogenates or plasma samples.

1) Plasma LH

Blood from each rat was collected using a modification of the method of Lushbough and Moline (191). With the etherized rat on its right side, a

flap was cut in the chest wall exposing the heart and descending aorta. The descending aorta was held with a pair of forceps and quickly dissected free from the dorsal wall with a blunt probe. After the aorta was cut on the distal side of the forceps, the central end was placed within a heparinized centrifuge tube and the occluding pressure exerted by the forceps released. The blood was centrifuged for 5-7 minutes, and the supernatant plasma was collected; this procedure generally yielded 2.5-4.5 ml of plasma. After its volume was recorded, the plasma was put into a second tube and quick-frozen in a dry ice-acetone bath. Samples were stored in the freezer until the day of assay (within three weeks of collection). Plasma was never pooled at the time of collection. Occasionally during the assay the minimum 2.0 ml needed was not available for a given injection; in such cases, enough plasma (0.05-0.25 ml) was taken from a similar sample to make up the difference; such cases are indicated in Appendix VI. In addition, plasma remaining after individual 2.0 ml samples were injected was occasionally pooled with like samples to permit an additional measurement to be made. Pooling was done only in these two special circumstances.

The plasma assay procedure consisted of removing the left ovary as a control, weighing it and placing it in 10 ml of 2.5% metaphosphoric acid. A 2.0 ml sample (saline, NIH-LH-S_{13}, or plasma) was immediately injected into the tail vein of the recipient rat under ether anesthesia. The right ovary was removed 1 ½ hours later, weighed and placed in another 10 ml volume of 2.5% metaphosphoric acid. The ovaries were homogenized and allowed to stand at least 10-15 minutes before being filtered. The supernatant was analyzed for ascorbic acid content using the spectrophotometric method of Midlin and Butler (208). From the ascorbic acid content and the ovarian weight, the ovarian ascorbic acid concentration (µg/mg ovary) was calculated. The per

cent ovarian ascorbic acid depletion (OAAD) was then calculated:

$$\frac{[OAA]_L - [OAA]_R}{[OAA]_L} \times 100\% = \%\ OAAD$$

$[OAA]_L$ = left ovary ascorbic acid (μg/mg)

$[OAA]_R$ = right ovary ascorbic acid (μg/mg)

% OAAD = per cent depletion in ascorbic acid

Plasma LH was estimated by comparing the per cent depletions of ovarian ascorbic acid due to the individual test plasma samples to a standard curve (determined with each assay) of the mean per cent depletions induced by five injections each of saline and three dilutions of a purified standard LH preparation supplied by the Endocrine Study Section of the National Institutes of Health (.0625, .25, and 1.00 μg, NIH-LH-S_{13}/2 ml). Because this method is not fully quantitative, each plasma LH value obtained was used as an independent data point even if the same plasma sample was used to obtain more than one data point. The data were then evaluated by calculating the mean and standard error, and comparing data between groups using Student's t-test for group comparison (significance at $p<.05$).

2) Pituitary LH

At autopsy pituitaries were removed by a ventral approach. The neurohypophysis was dissected off and discarded; the anterior pituitary was weighed and placed in a homogenizing tube and then frozen by placing the tube in a dry ice-acetone bath. The frozen pituitary was stored in the freezer until assay (within six months). Pituitaries were assayed individually, except during metestrus and estrus in the three-cycle study when, due to the low LH content, it was necessary to pool two pituitaries.

The pituitary assay procedure consisted of injecting, via the tail vein, saline or one of two concentrations of the pituitary homogenate having potencies approximating those of the two LH standard concentrations, 0.4 and 1.6 μg

NIH-LH-S_{13}/ml, which were also tested in each assay. Four hours after injecting the samples (n=5 for each treatment) the recipient rats were autopsied and the OAA concentration in the left ovary was determined as described above. The right ovary was usually discarded; in several assays it was kept to run a parallel check on the results calculated from the left ovary (the OAA concentration of the two ovaries is virtually identical).

The pituitary LH potency and 95% confidence limits were calculated using the statistical method and bioassay design of Bliss (25). Homogeneity or heterogeneity within a given group, or between groups, was tested by a Chi-Square analysis (25); the individual pituitary potencies within a given experimental group were then statistically combined to arrive at the weighted mean potency (25).

c. Functional state of neuroendocrine network

Vaginal cyclicity was evaluated in the two-month study by finding the average cycle lengths for the entire experimental period and comparing these in the control, sham, and lesioned animals. In addition, the two-month study was divided into two 30-day periods; the average cycle lengths of these two periods were then compared with each other and to their counterparts in the control, sham, or lesioned groups.

2. Ovarian Compensatory Hypertrophy Study

Immediately after lesioning or sham lesioning, the left ovary was removed and weighed; the ovary was then dried and reweighed. At autopsy, ten days later, the right ovary was removed, and wet and dry weights were obtained. The per cent difference of the right ovary compared to the left ovary was calculated according to the following formula:

$$\frac{W_R - W_L}{W_L} \times 100\% = OCH$$

W_R = weight of right ovary

W_L = weight of left ovary

OCH = per cent ovarian compensatory hypertrophy

The time course of the OCH response in controls was evaluated by constructing a control chart showing the degree of OCH at 0, 10, and 15 days after removal of the left ovary.

The average OCH and its standard error were calculated for each of the control, sham, and lesioned groups. Significance between these groups was then evaluated using Student's t-test for group comparison.

CHAPTER IV

RESULTS AND COMMENTS

A. The Three-Cycle Study

The data from the individual rats used in this study are tabulated in Appendices III, VI, and VIII. Lesion placement in these animals was very accurate, in most cases involving the medial-posterior portion of the ACO and some of the lateral-posterior aspect of the medial amygdaloid nucleus (AME) (Figure 2). In a few animals the lesion seemed confined to the posterior ACO with little or no involvement of the AME. The anterior-most part of the ACO was never involved in the lesions. Control animals in this study were 83 to 140 days of age at autopsy; the sham-operated rats were autopsied at 101 to 142 days of age, 11 to 37 days postoperative. The lesioned animals were 105 to 151 days old at autopsy, 11 to 42 days after the lesioning procedure. Although body weight (Table II) was somewhat depressed in the sham ($p<.01$) and lesioned ($p<.05$) animals, there were no overt signs of inadequate nutrition; while the animals' food intake was not closely watched, it was not obviously altered. General pituitary function had not been grossly disturbed, as indicated by the normal pituitary, thyroid, adrenal, and ovarian weights (Tables II and III).

1. Indirect Assessment of Circulating Ovarian Steroid Levels

a. Vaginal smear cycles

Following lesioning, animals either continued cycling or lapsed into a period of pseudopregnancy (leukocytic vaginal smears) lasting 12 to 15 days.

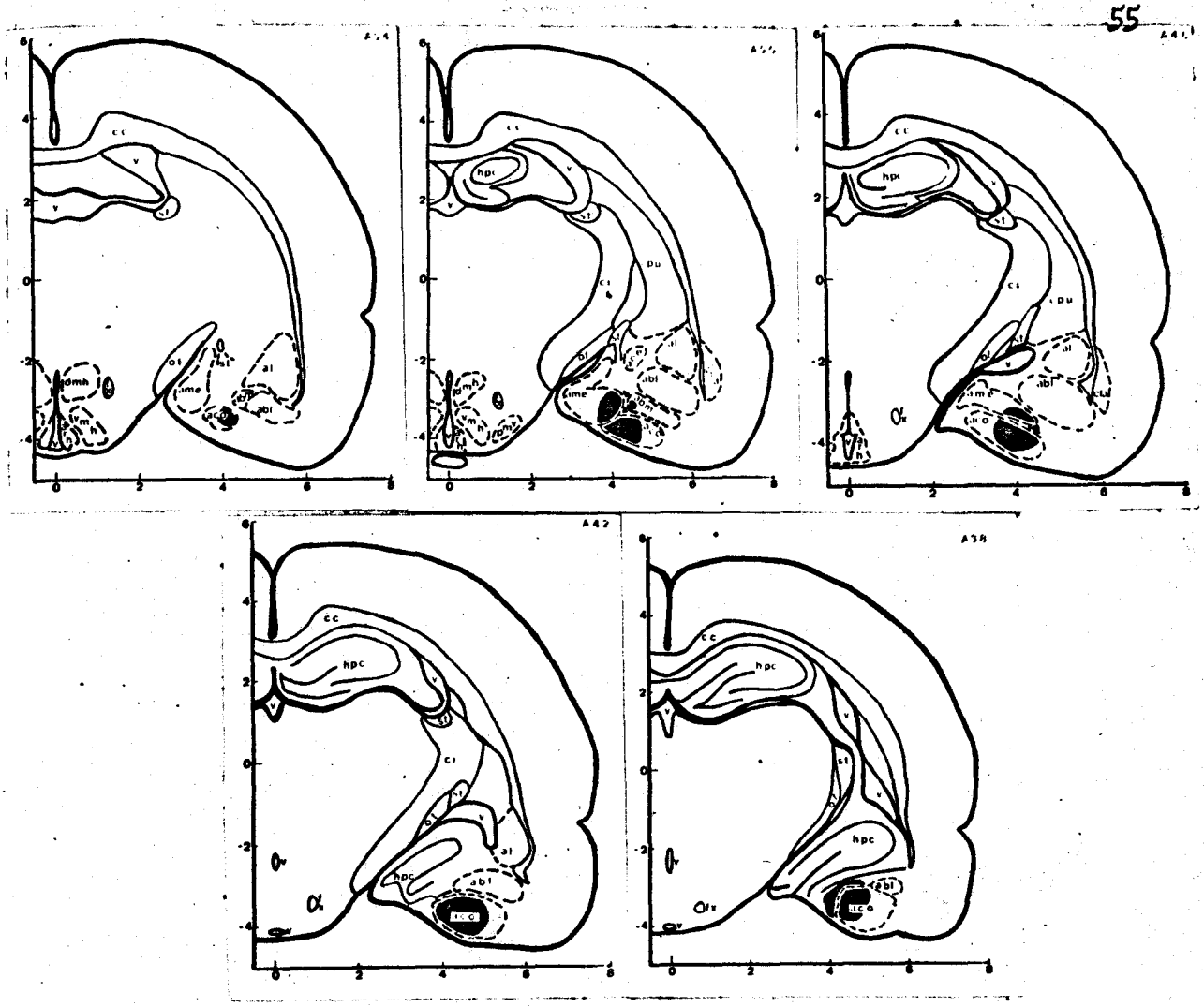


FIGURE 2.

Diagramatic Representation of the ACO Lesion Site.

abl - basolateral amygdaloid nucleus
abm - basomedial amygdaloid nucleus
ace - central amygdaloid nucleus
aco - cortical amygdaloid nucleus
al - lateral amygdaloid nucleus
ame - medial amygdaloid nucleus
arh - arcuate nucleus of the hypothalamus
cc - corpus callosum
ci - internal capsule
cla - claustrum
cpu - caudate nucleus and putamen
dmh - dorsomedial hypothalamic nucleus
fx - fornix
hpc - hippocampus
ot - optic tract
pmv - ventral premammilary nucleus
st - stria terminalis
v - ventricle
vmh - ventromedial hypothalamic nucleus

Modified from De Groot (60).

TABLE II

BODY AND THYROID WEIGHTS AND AUTOPSY AGES IN THE 3-CYCLE STUDY

	Body Weight (gm)	Thyroid Weight (mg)	Age at Autopsy (Days)	Days Postoperative
CONTROL	300±4 (41)† [ab]	15.9±0.6 (34)	115.8±1.7 (43)	------
SHAM-ACO	281±5 (26) [a]	17.7±0.9 (26)	119.8±2.1 (28)	18.2±1.5 (28)
LESION-ACO	286±5 (36) [b]	16.0±0.4 (36)	121.2±1.8 (36)	22.8±1.3 (36)

†Mean ± SEM (Number of Animals)

a $p<.01$

b $p<.05$

In this and all subsequent tables, means with similar superscripts are significantly different at the level indicated in the table footnotes.

TABLE III

PITUITARY, ADRENAL, AND OVARIAN WEIGHTS (mg) IN THE 3-CYCLE STUDY ANIMALS

	PROESTRUS	ESTRUS - AM	ESTRUS - PM	METESTRUS
		Pituitary Weights (mg)		
CONTROL	12.8±0.9 (10)†	14.1±0.8[a] (8)	12.6±0.9 (10)	13.1±0.7 (15)
SHAM	11.0±0.8[b] (7)	11.3±0.8[ac] (7)	14.3±1.0[bc] (7)	11.6±1.2 (7)
LESION	12.4±1.0 (9)	12.1±0.9 (7)	12.2±0.9 (10)	11.5±0.9 (10)
		Adrenal Weights (mg)		
CONTROL	29.4±2.2 (9)	31.7±2.1 (8)	29.6±1.3[d] (10)	31.5±1.2 (15)
SHAM	29.6±1.9[e] (7)	32.1±1.8 (7)	35.6±1.6[de] (7)	33.3±1.4 (7)
LESION	32.7±1.6 (9)	32.2±2.1 (7)	31.7±1.6 (10)	32.1±1.5 (10)
		Ovarian Weights (mg)		
CONTROL	37.6±3.0 (9)	35.5±1.4 (8)	39.2±2.3 (10)	39.1±1.2 (15)
SHAM	37.9±2.7 (7)	37.6±1.8 (7)	41.1±2.6 (7)	37.9±1.4 (7)
LESION	36.1±2.8 (9)	37.1±2.4 (7)	42.0±2.8 (10)	36.8±2.7 (10)

†Mean ± SEM (Number of Animals)

abce $p<.05$

d $p<.02$

No unusual numbers of cornified cells were noticed during the three cycles allowed before autopsy, indicating no marked changes in plasma ovarian steroids which would be reflected by increased incidence of vaginal cornification (88, 252).

b. Uterine changes

The uterine data summarized in Tables IV and V provide very reliable, although indirect, assessment of ovarian steroid levels during the estrous cycle (12, 13, 274). As reported previously by others (148, 248, 274), uterine weights fluctuate significantly during the estrous cycle; in the control animals uterine wet and dry weight is maximal at proestrus and minimal at metestrus (Table IV). By the afternoon of estrus, uterine wet weight had dropped significantly ($p<.01$) from the high proestrous weight; by metestrus a further drop occurred such that the metestrous-PM uterine weight was significantly lower ($p<.01$) than that at proestrus, estrus-AM, or estrus-PM. Although the dry weights did not drop significantly by estrus-PM, the metestrous dry uterine weight was significantly lower ($p<.01$) than at the three earlier time periods tested. The uterine tissue lost water between proestrus and metestrus. Compared to the proestrous values, the percent dry weight (the values in Table IV are the averages calculated from the individual values) became progressively greater during the cycle, estrus-AM ($p<.05$), estrus-PM ($p<.02$), and metestrus ($p<.01$). These measurements (drop in uterine weight and tissue fluid) indicate plasma estrogen levels have dropped between proestrus and metestrus (13, 274).

Although a general trend toward lower uterine weight and tissue fluid was apparent in the ACO-sham lesioned animals between proestrus and metestrus, a significant ($p<.02$) drop in wet weight from the proestrous high was not seen until metestrus, and no significant drop developed in the dry weights. The lack of an earlier significant drop in the wet weight and tissue fluid was

TABLE IV

UTERINE DATA IN THE 3-CYCLE STUDY

		PROESTRUS	ESTRUS - AM	ESTRUS - PM	METESTRUS
CONTROL	A.	525.6±29.1^{ab}(10)†	451.7±21.2^{c}(8)	427.4±12.1^{ad}(10)	332.1±14.0^{bcd}(15)
	B.	108.0±10.2^{m}(5)	92.7± 3.6^{n}(8)	88.5± 3.0^{o}(6)	72.9± 3.1^{mno}(13)
	C.	19.6± 0.4^{stu}(5)	20.6± 0.3^{svw}(8)	20.9± 0.2^{tx}(6)	21.6± 0.3^{uv}(13)
SHAM-ACO	A.	523.1±54.1^{e}(7)	443.7±18.2^{f}(7)	429.9±15.0^{g}(7)	353.1±27.9^{efg}(7)
	B.	83.3±11.5 (3)	87.8± 3.9 (6)	84.7± 3.4 (4)	76.3± 9.5 (4)
	C.	19.3± 0.8 (3)	19.4± 0.4^{wy}(6)	19.8± 0.3^{x}(4)	20.8± 0.2^{y}(4)
LESION-ACO	A.	502.6±19.7^{hij}(9)	431.4±23.3^{hk}(7)	424.9±24.7^{il}(10)	317.4±14.5^{jkl}(10)
	B.	91.9± 4.9^{p}(5)	88.4± 6.2^{q}(6)	88.3± 6.3^{r}(7)	68.0± 3.7^{pqr}(7)
	C.	19.2± 0.3$^{zq\phi}$(5)	20.3± 0.3$^{z\Delta}$(6)	20.3± 0.3$^{\sigma\nabla}$(7)	21.2± 0.3$^{\phi\Delta\nabla}$(7)

†Mean ± SEM (Number of Animals)

A. Wet Weight (mg) B. Dry Weight (mg) C. % Dry Weight

a-d,j-p,u,ϕ $p<.01$ e,f,q,r,t $p<.02$ g-i,s,v-z,$\sigma\Delta\nabla$ $p<.05$

TABLE V

UTERINE INTRALUMENAL FLUID AND NUMBER OF TUBAL OVA IN THE 3-CYCLE STUDY ANIMALS

	Uterine Intralumenal Fluid (mg)	Tubal Ova*	
	PROESTRUS	ESTRUS - AM	ESTRUS - PM
CONTROL	217.8±28.6 (9)	6.7±1.1 (8)	7.5±0.7 (10)
SHAM-ACO	252.2±32.4 (7)	6.1±0.9 (7)	7.3±0.3 (7)
LESION-ACO	236.1±56.7 (8)	5.6±0.4 (7)	7.1±0.7 (10)

†Mean ± SEM (Number of Animals)

*Number of ova expressed from dissected oviduct

probably due to the large variability associated with the proestrous wet weight and percent dry weight. Inspection of the individual data in Appendix III indicates the lack of a significant drop in dry weights was due to the low number of dry uterine weights which were unfortunately at the low end of the data population. However, these few animals may have had low tissue responsiveness to estrogen. Thus, the ACO-sham lesioned animals seem to have undergone a drop in plasma estrogen levels similar to that of the control animals. The significantly lower percent dry weights at estrus-AM and estrus-PM compared to their respective control values may indicate the estrogen drop was somewhat slower than in controls, or that progesterone levels were somewhat lower than controls. However, the normal loss of intralumenal fluid from sham animals indicates a rise of progesterone between proestrus and estrus closely resembling that in unoperated animals (more details below). That diestrous steroid levels inducing these uterine changes were close to control levels was indicated by the comparable proestrous uterine weights and tissue fluid (174, 248, 251) between control and sham animals.

The uterine wet weights underwent a very obvious decline in the lesioned animals, i.e., estrous-AM, estrous-PM, and metestrous animals all had uterine weights significantly lower than those at proestrus ($p \leq .05$, $p < .05$, $p < .01$, respectively). The metestrous uterine wet weight was also significantly lower ($p < .01$) than both the estrous-AM and estrous-PM weights. The dry weight data do not demonstrate as obvious a change as did the wet weights. No significant drop from proestrus occurred during estrus but there was a marked reduction in dry weights by metestrus-PM ($p < .01$). A significant difference between metestrus and estrus-AM ($p < .02$) or estrus-PM ($p < .02$) was seen in the dry weight data as well as in the wet weight data. A drop in estrogen titers was also reflected by the fact that the uterine tissue fluid was less, compared to that at

proestrus, during the estrous-AM ($p<.05$), estrous-PM ($p<.05$), and metestrous ($p<.01$) autopsy periods. In addition, at both estrus-AM and estrus-PM the uteri had more tissue fluid than at metestrus ($p<.05$). These data strongly indicate that plasma ovarian steroid levels throughout the cycle were very similar in both control and lesioned animals. The diestrous steroid levels inducing the uterine weight and tissue fluid changes (174, 248, 251) appear normal in lesioned animals, as does the change in circulating steroids during estrus and metestrus.

Table V summarizes the data on the uterine lumenal fluid content at proestrus. There was no statistical difference in the amount of fluid among the experimental groups. No lumenal fluid was found during either estrus or metestrus. It seems, therefore, that the diestrous steroid levels inducing the accumulation of lumenal fluid (12, 251), as well as the post-ovulatory steroid changes inducing loss of the lumenal fluid (12, 190) had not been seriously influenced by the lesioning procedure.

c. Pituitary weight

It is apparent from the pituitary data summarized in Table III that no weight changes occurred during the cycle in either the control or lesioned animals; nor were there any significant differences between control and lesioned animals' pituitaries on respective days of the cycle. Pituitary weight on the morning of estrus in the controls was significantly greater ($p<.05$) than that in the sham group autopsied at the same time, but virtually identical to that in the shams at estrus-PM. The elevated estrous-PM weight in the sham animals was significantly higher than both the proestrous ($p<.05$) and estrous-AM ($p<.05$) weights in the sham group. It is not known whether these differences reflect significant physiological changes. In that chronically high estrogen titers reportedly elevate pituitary weights (106), the

elevated pituitary weights may indicate high circulating estrogen levels at estrus. This is unlikely, however, since it is doubtful whether estrogen levels could change rapidly and greatly enough to induce the significant increases seen during estrus; furthermore the uterine weight and tissue fluid measurements (Table IV) indicate that highest estrogen levels were during proestrus.

d. Adrenal weight

Table III, section B, summarizes the adrenal data. Neither the control nor lesioned animals showed significant fluctuations during the estrous cycle. The sham animals, however, had a significantly greater ($p<.05$) estrous-PM adrenal weight compared to that at proestrus; it was also significantly greater than that in estrous-PM controls ($p<.02$). Lesioning did not induce any increase in adrenal weight; in that conditions of elevated estrogen have been reported to elevate adrenal weights (223), it is apparent that secretion of chronically high levels of estrogen have not been induced in lesioned animals. Even though the adrenal weights may indicate elevated estrogen levels in sham animals at estrus-PM, the more reliable uterine date indicate normal estrogen levels. The significance of the elevated adrenal weight is therefore doubtful.

2. Indirect Assessment of Circulating Gonadotrophin Levels

a. Ovarian weight

Table III summarizes the ovarian weight data. Examination of the control, sham, and lesion data reveals no significant fluctuations within any respective groups during the cycle. There were no statistically significant differences among the control, sham, or lesioned animals at respective cycle stages.

b. Ovulation

Table V summarizes the data on the number of tubal ova (unilateral) in estrous animals. No ova were found during other stages of the cycle. The number of eggs in the oviduct during estrus-AM compared to estrus-PM was not

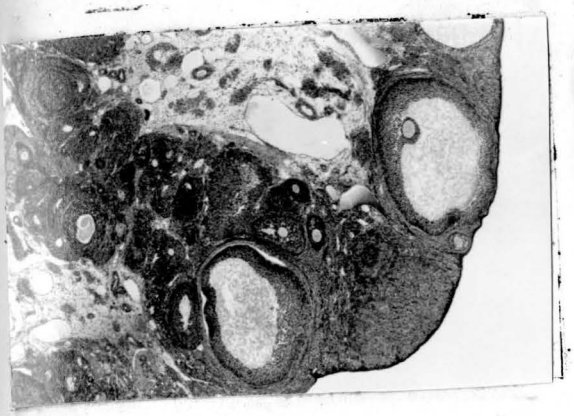
significant in any of the groups, nor was there any significant difference among groups during respective estrous time periods. It seems reasonable to conclude from these data that ACO-lesions did not induce marked changes in mean cyclic plasma gonadotrophin titers maintaining the normal ovarian weights, or in the basal and surge levels of plasma gonadotrophins needed for folliculogenesis, steroid secretion, and ovulation.(88).

c. Ovarian histology

Examination of the ovarian histology revealed that full luteinization apparently did not occur in the ACO-lesioned animals. In control animals, large follicles were occasionally seen at estrus but they were never comparable in size or number to follicles found at proestrus (Figure 3); the center of many fresh corpora lutea was not densely infiltrated by cells. Fresh eggs were visible in the ampullar segment of the oviduct (Figure 3). At metestrus follicular development is noted and all corpora lutea were solid (Figure 3). The ovaries of sham or lesioned animals during estrus contain several stimulated follicles comparable to those seen during proestrus (Figures 4 and 5). During metestrus, sham ACO lesioned animals have corpora lutea much like those of control animals (Figure 4). In lesioned animals however, although cellular infiltration of the corpora lutea is underway, many corpora lutea still retain a distinct fluid center (Figure 5). Some of the larger follicles remaining through estrus in lesioned animals apparently may become luteinized, trapping the ova within (Figure 5). These histological findings suggest that plasma gonadotrophin concentrations during the cycle may be inadequate for normal luteinization.

3. Direct Assessment of Plasma and Pituitary LH

Table VI summarizes the plasma LH data. The data points in Appendix VI are given in the units directly found from the assay (μg/2 ml) and the converted



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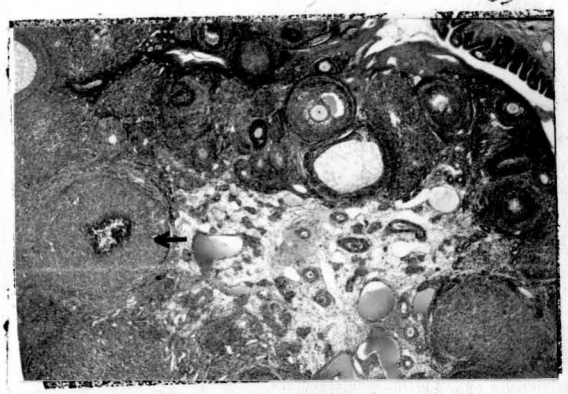

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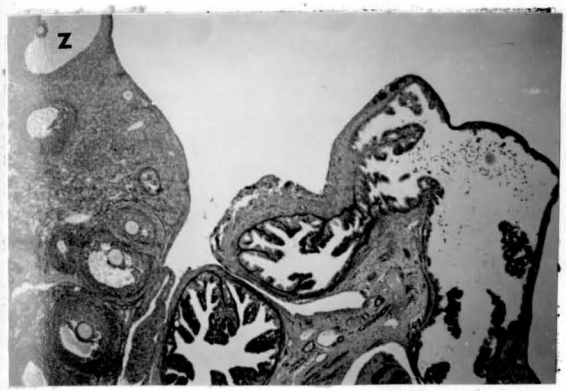

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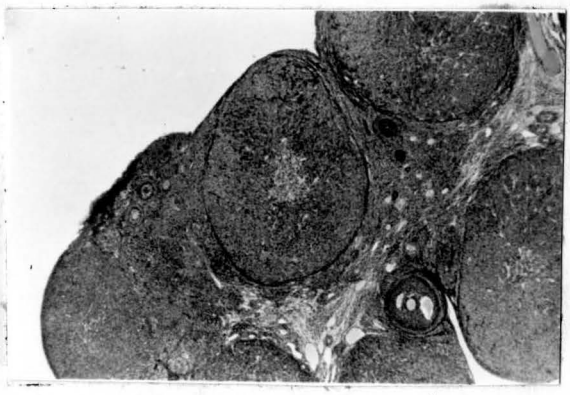


D.

FIGURE 3

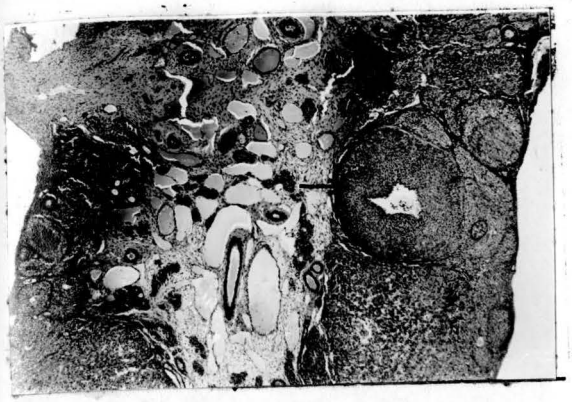
Sections of Ovaries from Control 3-Cycle Study Animals. 80X.

A. Proestrus. Two developed follicles. Animal SB8.

B. Estrus. Fresh corpus luteum. Animal SB37.

C. Estrus. Egg in oviduct (x) and developing follicle (z). Animal SB37.

D. Metestrus. Corpus luteum which looks most like those found in sham or lesioned animals. Animal SB22.



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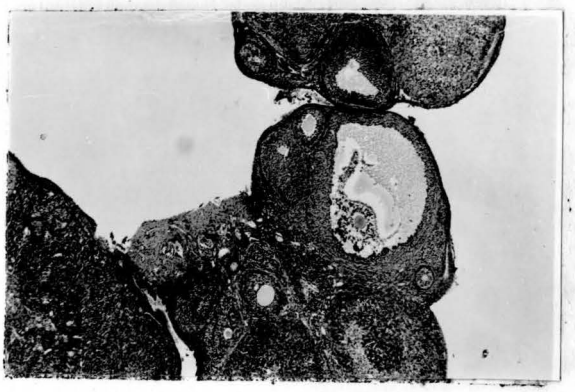

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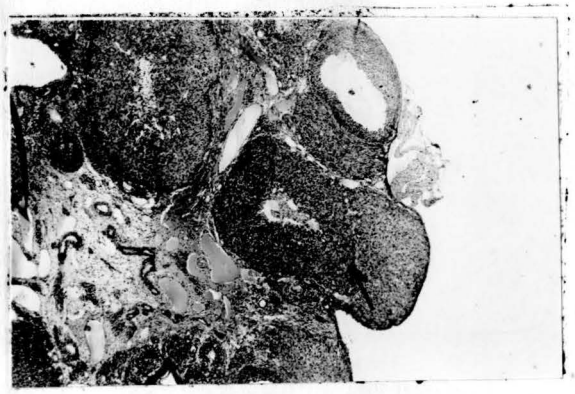


C.

FIGURE 4

Sections of Ovaries from ACO-Sham Operated Animals in the 3-Cycle Study. 80X.

A. Estrus. Fresh corpus luteum. Animal SB34.

B. Estrus. Highly stimulated follicle; luteinization is evident and ovum and surrounding granulosal cells are free within follicular fluid. Animal SB37.

C. Metestrus. Fresh corpus luteum. Animal SB15.

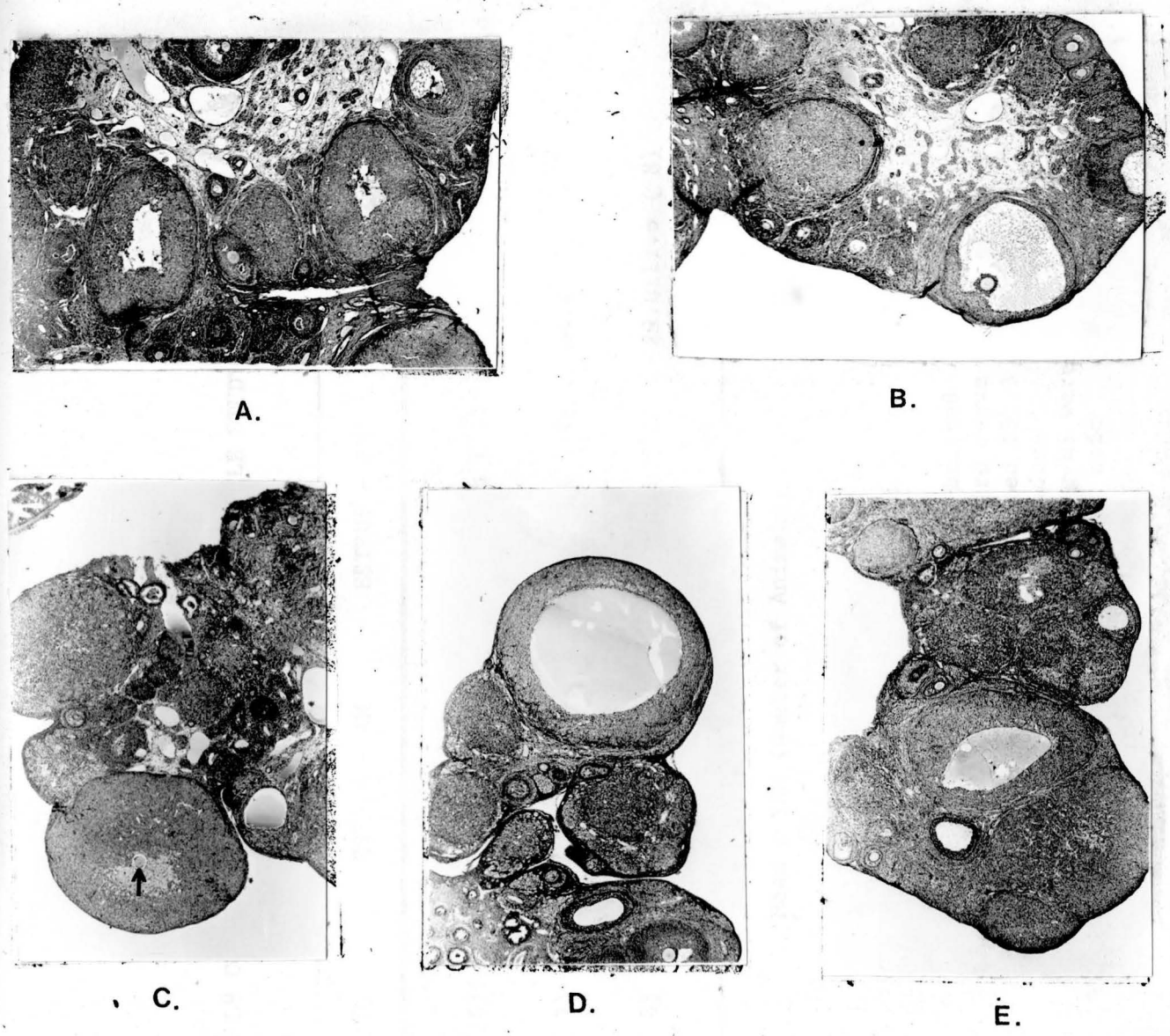


FIGURE 5

Sections of Ovaries from ACO-Lesioned Animals in the 3-Cycle Study. 80X.

A. Estrus. Two fresh corpora lutea, each with lumen. Animal SB25.

B. Estrus. Large stimulated follicle comparable to those seen during proestrus. Animal SB25.

C. Metestrus. Corpus luteum with entrapped ovum. Animal SB18.

D. Metestrus. Corpus luteum with large lumen. Animal SB18.

E. Metestrus. Corpus luteum with large lumen. Animal SB35.

TABLE VI

PLASMA LH CONCENTRATIONS (ng/ml) IN THE 3-CYCLE STUDY

	PROESTRUS	ESTRUS - AM	ESTRUS - PM	METESTRUS
CONTROL	32.2±5.9[ab](16)†	10.8±3.6[ac](9)	9.5± 3.8[bde](9)	40.9±10.5[cd](13)
SHAM-ACO	24.1±7.4*(7)	11.2±7.4 (5)	26.6±6.8[e](6)	54.1±22.6 (5)
LESION-ACO	24.3±8.3[f](8)	5.6±2.4[f](8)	26.9±11.1 (11)	29.0±17.6 (8)

†Mean ± SEM (Number of Animals)

ab $p<.02$

c-f $p<.05$

*Values below 31.3 ng/ml were obtained by extrapolation of the standard curve below the lowest standard tested (31.3 ng/ml), assuming a linear log-dose response down to zero. Zero ng/ml were assumed if no ovarian ascorbic acid depletion occurred.

units used in Table VI (ng/ml). Plasma LH during the experimental autopsy period (2:30-3:30 PM) in proestrous control animals was significantly less ($p<.01$) than that found during the 5:00-6:00 PM time period [201.3±18.5 (SEM) ng/ml (seven data points) (Appendix VI)]. Since the latter was obtained using five-day animals and, since most experimental animals were four-day cyclers, this may not be the best comparison (248). Using four-day proestrous animals from the same supplier as used in the present study, Lawton (173) has recently noted a plasma LH concentration of 61.3±12.6 ng/ml (191) between 6:00 and 7:00 PM and a concentration of 84.5±23.2 ng/ml (199) between 8:00 and 9:00 PM, indicating that the peak plasma LH concentration occurred later than the 2:30-3:30 PM time period noted in Sprague-Dawley rats from another supplier (176). The experimental autopsy time used for this study was thus apparently several hours earlier than the true critical period. Nevertheless, as seen in Table VI, LH release has already begun, in that plasma LH at this time is significantly higher ($p<.02$) than values found during the morning and afternoon of estrus. One unexpected finding was the significantly elevated plasma LH concentration in metestrous controls compared with that found during estrus ($p<.05$); the value is not significantly different from that at proestrus.

In the sham-lesioned animals, plasma LH was high at proestrus, as in controls, but failed to show a significant drop by the morning of estrus, even though estrous-AM levels were similar to those in controls. Failure to demonstrate significance was probably due to the greater variability and smaller sample number. The estrous-PM concentration, while statistically higher ($p<.05$) than the control estrous-PM value, was not statistically higher than the sham estrous-AM value. The sham estrous-PM and proestrous concentrations seemed identical. It thus appears that the plasma LH titers may be slightly elevated during the afternoon of estrus in the sham-lesioned animals.

The lesioned animals demonstrated a significant ($p<.05$) drop in plasma LH between proestrus-PM and estrus-AM. Although estrous-PM and metestrous concentrations appear identical to those at proestrus, they are not significantly higher than the value at estrus-AM. Failure to demonstrate significance is probably due to the wide variability. The metestrous value, although lower than both control and sham values, is not significantly lower.

Although the statistics do not bear out a consistent trend, these data offer suggestive evidence that plasma LH was elevated on the afternoon of estrus, but not on the morning of estrus or on the afternoon of metestrus, in sham and lesioned animals. There is a slight indication in the data from the metestrous animals that the sham procedure may elevate plasma LH, but that the lesion may reduce the plasma LH during metestrus, although there was not statistical evidence for this separation. The morning-afternoon differences in the sham and lesioned animals may be better detected with more time periods evaluated and an assay method which allows less variability. These data also indicate that the LH surge had not been advanced by either the sham or lesioning procedures to such an extent that it could be detected by the 2:30-3:30 PM autopsy period.

The weighted mean pituitary LH potencies during the various stages of the estrous cycle are presented in Table VII; the individual pituitary LH values are tabulated in Appendix VII. Pituitary LH content in control animals dropped from a high at proestrus-PM to a low at estrus-AM ($p<.01$), and remained significantly lower ($p<.01$) through metestrus-PM. Contents on the morning and afternoon of estrus were virtually identical. While replenishment of pituitary LH seemed to occur by metestrus-PM, not enough of a recovery had been accomplished to yield a significant increase compared to estrus-AM. Sham and lesioned animals also showed a sharp drop in pituitary LH content from proestrus-PM to estrus-AM ($p<.01$). In both groups, however,

TABLE VII

PITUITARY LH POTENCY 3 CYCLES POST-LESION

	PROESTRUS	ESTRUS		METESTRUS
	PM	AM	PM	PM
CONTROL	24.6*[ab] (18.6 - 32.4)† 3‡	8.5[a] (6.8 - 10.6) 3	9.5[be] (7.2 - 12.5) 3	11.8 (8.8 - 15.8) 3
ACO-SHAM	18.6[c] (15.3 - 22.7) 3	9.2[c] (7.6 - 11.1) 3	13.4 (10.8 - 16.7) 3	13.4 (10.6 - 17.1) 3
ACO-LESION	22.0[d] (17.3 - 27.9) 3	12.0[d] (9.7 - 14.8) 3	17.8[e] (14.2 - 22.3) 4	16.8 (13.9 - 20.2) 3

*Weighted Mean Potencies [μg(NIH-LH-S_{13})/pituitary]

†95% Confidence Limits

‡Number of Pituitaries Combined

[a-e]Potencies Significantly Different ($p<.01$)

estrous-PM and metestrous levels were no longer significantly lower than their respective proestrous values; neither were they significantly higher than their respective estrous-AM levels. Thus, in both sham and lesioned animals, pituitary LH seems to be increased to somewhere between the proestrous high and the estrous-AM low by estrus-PM and remains there into metestrus-PM. The accumulation of pituitary LH seems to have occurred more efficiently or more rapidly in the lesioned animals, since the content at estrus-PM was significantly higher than in the controls (Table VII). The sham estrous-PM content appears to be somewhere in between the control and lesion contents; it was not significantly different from either of them. To further test the difference between sham and lesion animals, pituitary LH content for all estrous and metestrous animals were pooled within a given experimental group (control, sham, and lesion) and the potencies evaluated for statistical significance (Figure 6). The results of this procedure emphasize the more rapid accumulation of LH in the pituitaries of the ACO-lesioned animals than in control or sham-lesioned animals. The combined estrous-metestrous content was significantly greater ($p < .05$) in the ACO-lesioned animals than in either control or sham animals; however, pituitary LH in the control and sham groups was similar. It thus appears that the pituitaries of ACO-lesioned animals had an increased rate of replenishment of LH as compared to control or sham animals. Although ACO-lesioning encouraged a more rapid accumulation of pituitary LH, the presence of the lesion did not alter the maximum level attained prior to the ovulatory discharge of gonadotrophins.

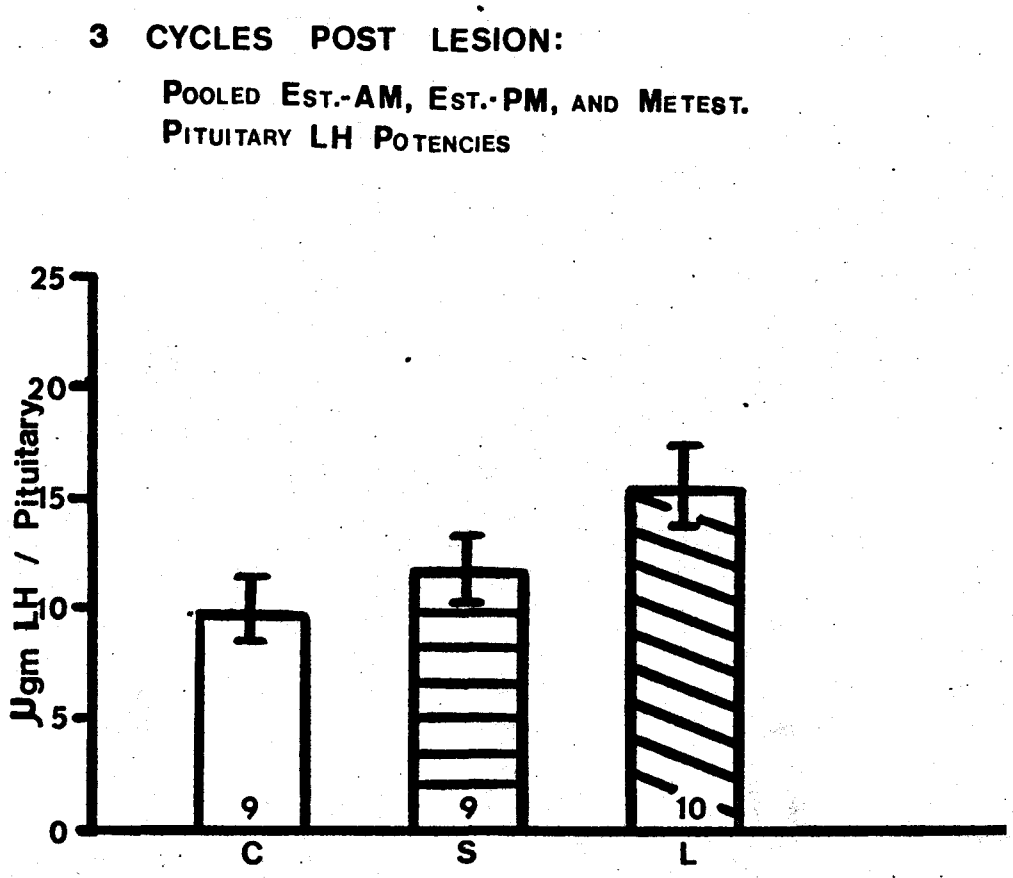


FIGURE 6

The weighted mean potency and 95% confidence limits (25) of LH in pituitaries of control (C), sham-lesioned (S), and ACO-lesioned (L) rats autopsied at estrus and metestrus.

B. The Two-Month Study

The data from each rat used in this study are compiled in Appendices I, IV, VI, and VII. Placement of lesions in the ACO was very accurate. For the most part, significant damage was done to the ACO with some encroachment on the medial nucleus (AME) (Figure 2); in a few cases, the lesion seemed confined to the ACO with little or no encroachment on the AME. The lesions were confined to the posterior portion of both the ACO and AME, with no obvious damage to the anterior part of the ACO or the AME. Lesion size ranged from 0.75 mm-1.75 mm. Lesions directed at the ST were in all cases inadequate. In no case was bilateral damage inflicted. The placements were 0.5-1.00 mm too ventral, such that even the spread of tissue damage out from the major lesion site did not generally reach the ST. Instead, the caudate nucleus, putamen, and internal capsule received various amounts of damage. Lesion placements in the AL, while not being uniformly direct, always involved the lateral portion of the nucleus along with extensive damage to the claustrum.

That the general health of the lesioned animals in the present study did not deteriorate was judged from their good general appearance and from the maintained body and organ weights (Tables VIII - XII). Body weights (Table VIII) were similar in 7 of the 8 groups studied; the ACO-lesioned rats were significantly heavier than their sham controls. Table IX summarizes the pituitary weights as a function of the cycle stage at autopsy. The weights do not vary in any group except the AL-sham group; since controls, ACO-shams or AL-lesion animals did not show this significant ($p<.05$) rise in pituitary weight between proestrus and estrus, its physiological significance is questionable. The significantly ($p<.02$) lower AL-sham proestrous pituitary weight compared to controls, and the lower estrous-PM value compared to both the control ($p<.05$) and ACO-lesion ($p<.05$) groups are likewise doubtful. It is possible, however,

TABLE VIII

BODY WEIGHT, AND AGE AT AUTOPSY OF THE 2-MONTH STUDY ANIMALS

		BODY WEIGHT (gm)	AGE AT AUTOPSY (days)	DAYS POST-OPERATIVE
CONTROL	(26)‡	312±4†	150.5±2.1	----
ACO-SHAM	(27)	310±3[a]	148.8±1.4	61.0±0.7
ACO-LESION	(28)	322±5[a]	149.9±1.6	60.4±0.6
ST-SHAM	(9)	315±11	154.9±0.8	61.6±0.5
ST-LESION	(15)	319±5	147.6±1.4	60.9±0.4
AL-SHAM	(12)	314±5	163.7±1.2	61.7±0.6
AL-LESION	(8)	313±13	160.7±0.7	60.7±0.7

†Mean ± SEM

‡Number of Animals

[a] $p < .05$

TABLE IX

PITUITARY WEIGHTS (mg) IN THE 2-MONTH STUDY ANIMALS

	PROESTRUS	ESTRUS - AM	ESTRUS - PM
CONTROL	16.5±0.6[a] (8)†	16.1±1.2 (9)	17.5±0.9[b] (9)
SHAM-ACO	16.0±0.8 (7)	16.4±0.7 (9)	15.0±0.7[bc] (11)
LESION-ACO	15.3±1.0 (10)	16.3±1.0 (8)	17.9±1.0[c] (10)
SHAM-ST	-----	16.0±1.1 (9)	-----
LESION-ST	16.0±1.1 (5)	16.3±0.5 (5)	16.5±1.5 (5)
SHAM-AL	13.4±0.9[ad] (5)	18.4±1.6[d] (6)	-----
LESION-AL	14.6±1.2 (5)	15.9±1.8 (4)	-----

†Mean ± SEM (Number of Animals)

[a] $p<.02$

[bcd] $p<.05$

that the sham procedures may have had some undisclosed unique effects of their own.

Thyroid weights (Table X) did not vary significantly during the estrous cycle in control, ACO-lesioned, AL-sham, or AL-lesioned animals. In the ACO-sham and "ST"-lesioned animals, however, thyroid weights at proestrus were significantly ($p<.05$) greater than at estrus. The significance of these variations, especially those of the latter group, is unclear since "ST"-lesions involved the caudate, putamen, and internal capsule. Thyroid weight at proestrus in the ACO-sham group was greater than that in the control ($p<.05$), AL-sham ($p<.05$), and AL-lesion ($p<.01$) groups. In the ACO-lesion group thyroid weight was significantly greater ($p<.05$) than in animals with AL-lesions at both proestrus and estrus; although it also appeared to be greater than that in controls, the difference fell short of significance. The significantly divergent effects of ACO and AL lesions on thyroid weight suggest opposing roles for these two amygdalar sites in the regulation of thyroid stimulating hormone (TSH) secretion.

Adrenal weights (Table XI) did not show any significant variation during the estrous cycle in any group except the ACO-lesion group, in which the adrenals were significantly heavier ($p<.01$) on the afternoon of estrus than in the morning. The estrous adrenal weights in the ACO-lesion animals were not significantly different from those in controls. However, this estrous-PM adrenal weight was also significantly higher than that in the shams ($p<.02$), whereas the estrous-AM weight was significantly lower than the sham ACO weight ($p<.05$). Although the physiological significance of the 25% increase in adrenal weight over a $3\frac{1}{2}$ hour period in the lesioned rats is not apparent at the present time, it may reflect a marked elevation in the circadian peak of adrenocorticotrophic hormone (112), or an elevation in circulating

TABLE X

THYROID WEIGHTS (mg) IN THE 2-MONTH STUDY ANIMALS

	PROESTRUS	ESTRUS - AM	ESTRUS - PM
CONTROL	16.5±1.3[a] (8)†	16.5±1.0 (9)	17.6±1.5 (9)
SHAM-ACO	19.8±0.5[abcd] (7)	17.1±1.0[b] (9)	16.6±0.9[c] (11)
LESION-ACO	19.1±1.0 (10)[f]	18.2±0.7 (8)[g]	17.7±0.8 (10)[h]
SHAM-ST	-----	16.1±2.3 (8)	-----
LESION-ST	18.4±1.1 (5)[i]	16.8±0.5 (5)	14.5±1.0 (5)[hi]
SHAM-AL	17.9±0.6[de] (5)	18.4±1.6 (6)	-----
LESION-AL	15.5±0.7[ef] (5)	14.7±1.3 (4)[g]	-----

†Mean ± SEM (Number of Animals)

[a-i] $p<.05$

$p<.01$

TABLE XI

ADRENAL WEIGHTS (mg) IN THE 2-MONTH STUDY ANIMALS

	PROESTRUS	ESTRUS - AM	ESTRUS - PM
CONTROL	27.5±1.2 (8)†	29.1±1.8 (9)	30.8±2.3 (9)
SHAM-ACO	29.4±1.0 (7)	29.7±0.9 (9)[a]	29.1±0.8 (11)[c]
LESION-ACO	30.3±1.4 (10)	26.4±1.3 (8)[ab]	32.9±1.3 (10)[bc]
SHAM-ST	-----	32.8±1.9 (9)[d]	-----
LESION-ST	28.5±1.5 (5)	26.9±1.1 (5)[d]	30.8±3.4 (5)
SHAM-AL	29.7±1.8 (5)	30.2±2.8 (6)	-----
LESION-AL	25.8±1.8 (5)	28.3±1.9 (4)	-----

†Mean ± SEM (Number of Animals)

[ad] $p<.05$

[b] $p<.01$

[c] $p<.02$

estrogens (223).

1. Indirect Measures of Plasma Ovarian Steroids

a. Vaginal smears

Estrogen levels were not markedly decreased or increased since estrous cycles proceeded normally (Table XII; Appendix I) and the incidence of cornified smears did not differ from normal. Full vaginal cornification was found during only one day of the cycle, estrus.

b. Uterine weights and lumenal fluid

Tables XIII and XIV summarize the uterine weight, tissue fluid, and intralumenal fluid data. The original data are tabulated in Appendix IV. As can be seen from Table XIII, both wet and dry weights in control animals on the afternoon of proestrus were significantly higher than on either the morning or afternoon of estrus. The uterine tissue fluid (as measured by the percent dry uterine weight) had also significantly dropped from proestrous levels during estrus. These uterine indicators, reflected a similar dynamic change of plasma estrogens in ACO-sham-lesioned animals, with the possible exception of a slower drop in uterine tissue fluid. A significant drop was not evident until estrus-PM, although both the wet and dry weights were significantly lower by estrus-AM. Animals bearing ACO-lesions also demonstrated the expected drop in uterine wet weight from the high proestrous level by both estrus-AM and -PM, Although the dry weights were lower at estrus than at proestrus, the difference was not significant. Uterine tissue fluid in the lesioned rats had dropped significantly by the morning of estrus, but then rose again by estrus-PM; estrous afternoon levels, statistically equivalent to proestrous levels, were significantly higher than morning levels and sham estrous-PM levels. These differences may reflect subtle alterations in estrogen and/or progesterone secretion during the cycle (13). However, since

TABLE XII

AVERAGE CYCLE LENGTH FOR RATS IN THE 2-MONTH STUDY

	0-30 Days Post Lesion	31-60 Days Post Lesion	0-60 Days Post Lesion
CONTROL	†4.08±0.03(29)[abcd]	4.28±0.07(29)[a]	4.18±0.04(29)
SHAM-ACO	4.24±0.05(30)[b]	4.26±0.08(30)	4.23±0.05(30)
LESION-ACO	4.16±0.04(30)	4.21±0.06(30)	4.17±0.04(30)
SHAM-ST	4.62±0.11[c](10)[e]	4.41±0.22(10)	4.40±0.15(10)
LESION-ST	4.19±0.08(15)[e]	4.18±0.08(15)	4.21±0.08(15)
SHAM-AL	4.28±0.10(12)[d]	4.19±0.11(12)	4.20±0.09(12)
LESION-AL	4.09±0.04(10)	4.11±0.10(10)	4.11±0.06(10)

†Mean ± SEM (Number of Animals)

[ad] $p<.02$

[bce] $p<.01$

TABLE XIII

UTERINE DATA IN THE 2-MONTH STUDY ANIMALS

	PROESTRUS	ESTRUS - AM	ESTRUS - PM
CONTROL	635.4±40.3^{ab}(8)*[1]	494.6±28.6^{a}(9)	503.6±37.6^{b}(9)
	114.8± 6.4^{gh}(8)[2]	94.6± 5.5^{g}(9)	95.5± 5.8^{h}(9)
	18.1± 0.2^{kl}(8)[3]	19.1± 0.3^{k}(9)	19.1± 0.3^{l}(9)
ACO-SHAM	763.9±67.9^{cd}(7)	543.8±32.9^{c}(9)	507.8±31.2^{d}(11)
	139.3±13.1^{ij}(7)	101.5± 5.3^{i}(9)	98.8± 5.8^{j}(11)
	18.2± 0.3^{m}(7)	18.8± 0.4 (9)	19.5± 0.2^{mn}(11)
ACO-LESION	622.0±33.0^{ef}(10)	505.1±40.1^{e}(8)	524.6±21.4^{f}(10)
	113.2± 6.9 (10)	99.5± 7.9 (8)	98.4± 4.5 (10)
	18.2± 0.3^{o}(10)	19.7± 0.2^{op}(8)	18.7± 0.2^{npq}(10)
ST-SHAM	---	536.6±39.2 (9)	---
	---	100.2± 6.8 (9)	---
	---	18.7± 0.8 (9)	---
ST-LESION	590.8±48.8 (5)	494.4±40.4 (5)	451.7±44.5 (5)
	108.6± 8.9 (5)	98.8± 6.8 (5)	90.1± 8.6 (5)
	18.4± 0.1^{rs}(5)	20.3± 0.3^{r}(5)	20.0± 0.4^{qs}(5)
AL-SHAM	619.3±43.7 (5)	502.5±48.8 (7)	---
	113.5± 6.8 (5)	95.8± 8.7 (7)	---
	18.4± 0.4 (5)	19.1± 0.3 (7)	---
AL-LESION	594.7±42.3 (5)	464.2±46.7 (3)	---
	105.4± 9.5 (5)	93.8± 8.6 (3)	---
	17.6± 0.3 (5)	20.2± 0.3 (3)	---

*Mean ± SEM (Number of Animals)

[1]Uterine Wet Weight (mg)

[2]Uterine Dry Weight (mg)

[3]Percent Uterine Dry Weight

p<.01: cdijmoqrst

p<.02: anp

p<.05: befghkl

TABLE XIV

UTERINE INTRALUMENAL FLUID AND NUMBER OF EGGS

IN THE 2-MONTH STUDY ANIMALS

	Uterine Intralumenal Fluid (mg)	Number of Eggs*	
	PROESTRUS	ESTRUS - AM	ESTRUS - PM
CONTROL	274.8± 32.5 (8)†	6.3±0.7 (9)	6.7±0.5 (9)
SHAM-ACO	605.9±194.3 (7)	5.9±0.8 (9)	6.9±0.7 (11)
LESION-ACO	238.1± 46.5 (9)	4.9±0.8[a] (8)	7.9±0.7[a] (10)
SHAM-ST	-----	6.2±0.6 (9)	-----
LESION-ST	347.3± 83.9 (5)	6.6±0.7 (5)	6.2±0.8 (5)
SHAM-AL	457.6±118.2 (5)	7.7±0.5 (6)	-----
LESION-AL	332.7± 69.2 (5)	7.0±1.2 (4)	-----

†Mean ± SEM (Number of Animals)

[a] $p<.02$

*Except for animal SD32 in which no ova were found in the right duct, one ova found between the ovary and the capsule in the histological sections of the left ovary and used for the ova count of this animal, the number of ova expressed from the dissected right oviduct were used.

proestrous and estrous wet and dry weights and tissue fluid in the sham and lesioned groups were equivalent to control values, plasma estrogen levels apparently were physiologically equivalent.

In the "ST"-lesioned animals, uterine wet and dry weights at proestrus and estrus were similar to those in controls. Although a significant decrease of tissue fluid was evident between proestrus and both estrus-AM and estrus-PM, the proestrus-to-estrus drop in wet and dry weights was not significant, perhaps because of the small population of animals (225).

The findings of the AL study closely parallel those of the "ST"-study. Although there were no differences in uterine wet or dry weights in the sham or lesioned rats when compared with controls, the difference between the proestrous and estrous-AM uterine wet and dry weights was not significant. Again, however, there was a significant reduction in uterine tissue fluid between proestrus and estrus-AM, with the respective values being indistinguishable from their control values.

The accumulation of intralumenal fluid was not significantly disturbed by any of the sham or lesion procedures (Table XIV). Lumenal fluid was found only during proestrus. This indicates that 1) relatively normal low progesterone plasma levels were present prior to and during early proestrus, allowing the accumulation of uterine lumenal fluid, and 2) a relatively normal increase of progesterone occurs between proestrus-PM and estrus-AM (12).

c. Pituitary weights

Pituitary weights (Table IX) offer further evidence that estrogen levels were not grossly altered. In that estrogen elevates pituitary weights (106), if estrogen levels were elevated throughout the estrous cycle after lesioning, one would expect higher pituitary weights than in controls. Such an increase was not seen for any experimental group.

2. Indirect Measures of Gonadotrophin Secretion

a. Ovarian weights

Inspection of the ovarian weight data (Table XV) reveals no significant fluctuations between proestrus and estrus in the control, the ACO-, "ST"-, or AL-lesion, or AL-sham group. Only the ACO-sham group had a lower mean ovarian weight at proestrus than at either estrus-AM or estrus-PM, and any physiological significance is unlikely. Comparison of the control, ACO-lesion, and ACO-sham data reveals that two months after lesioning, ovarian weights, and thus plasma gonadotrophin levels, have not been shifted. Ovarian weights in the "ST" and AL groups were also statistically indistinguishable from those in the controls. Therefore, a gross shift in plasma gonadotrophin concentrations during the estrous cycle did not appear to exist two months after lesioning.

b. Vaginal smear cycles

Vaginal smear cyclicity was not obviously altered by the lesions (Table XII; Appendix I), offering additional evidence that plasma gonadotrophin levels were not grossly altered.

The average cycle length of the ACO-lesioned animals was statistically comparable to both the control and ACO-sham lesioned animals during the first and second thirty day periods of the experiment, as well as the entire 60-day experimental period. Although the control animals developed a significant shift toward five-day cycles in the second half of the experimental period, none of the other experimental groups developed this shift in average cycle length. Since virtually all operated animals showed either a period of pseudopregnancy (12-16 days of diestrous vaginal smears) which was discounted in figuring the average cycle lengths, or a lengthening of the cycle immediately after lesioning from the previous four to a five-day cycle, it is felt the somewhat longer cycles during the first thirty days were a reflection of

TABLE XVI

OVARIAN WEIGHTS (mg) IN THE 2-MONTH STUDY ANIMALS

	PROESTRUS	ESTRUS - AM	ESTRUS - PM
CONTROL	36.2±2.2[a] (8)†	39.4±2.7 (9)	36.3±1.6 (9)
SHAM-ACO	29.5±2.0[abcd] (7)	38.3±1.2[d] (9)	38.3±2.9[c] (10)
LESION-ACO	36.1±1.5[b] (10)	36.0±2.3 (8)	39.8±2.7 (10)
SHAM-ST	[illegible]	36.8±2.0 (9)	-----
LESION-ST	38.8±2.7 (5)	36.7±2.1 (5)	32.6±3.2 (5)
SHAM-AL	37.3±5.9 (5)	33.9±1.5 (7)	-----
LESION-AL	33.7±2.3 (5)	37.7±2.8 (3)	-----

†Mean ± SEM (Number of Animals)

[ac] $p<.05$

[b] $p<.02$

[d] $p<.01$

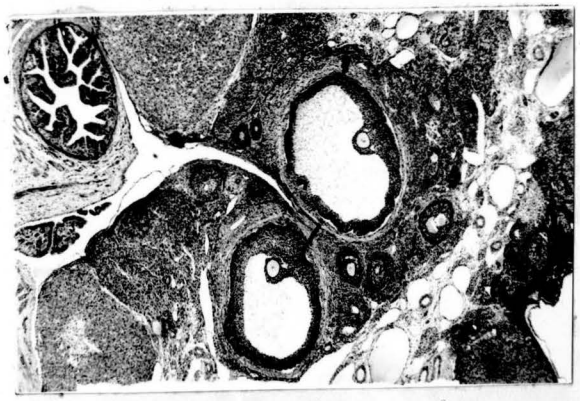
the operative trauma. The average cycle length during the entire 60-day period in the experimental animals shows no tendency to differ from controls. Maintenance of estrous cycles following ACO and AL lesions indicates that the amygdala regions lesioned are not essential CNS components for the maintenance of the estrous cycle or for establishment of the period of the cycle.

c. Ovulation

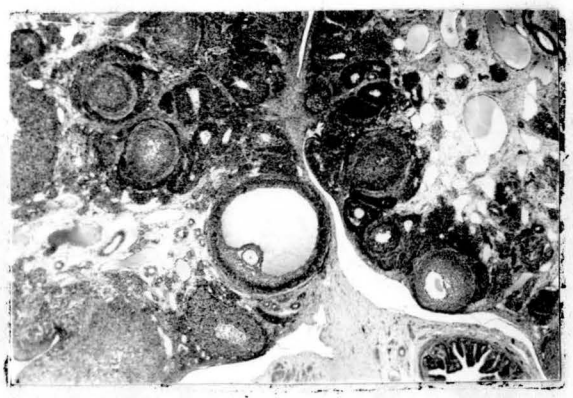
Although the comparable number of tubal ova in the control, sham-lesioned, and lesioned animals (Table XIV) by the afternoon of estrus further substantiates equivalent plasma gonadotrophin levels among the various groups, the significantly lower number of eggs at estrus-AM compared with estrus-PM in ACO-lesioned animals suggests a delay in the ovulation process. The lack of such a difference in the ACO-sham or in the "ST"-lesioned group indicates the delay could not be attributed to nonspecific brain damage following electrode placement or lesioning. In that the number of eggs at estrus-AM was comparable between control and ACO-lesioned animals, an actual delay in ovulation is not definite without further evidence (see below - Ovarian histology).

d. Ovarian histology

Ovarian follicles at estrus in control animals do not compare in either size, general shape, or number with the large stimulated follicles seen during proestrus (Figure 7). In the sham ACO-lesioned estrous animals most of the follicles observed were similar to those in the controls (Figure 8); however, a few larger proestrous-type follicles were also found (Figure 8). In the ACO-lesioned estrous animals these large stimulated follicles seemed more prevalent (Figure 9); nevertheless, new corpora lutea and tubal ova were also found in the same ovaries. Occasionally heavily luteinized follicles were found which contained entrapped ova (Figure 9), surrounded by granulosa cells and floating free within the follicular fluid. In no case was such extensive



A.



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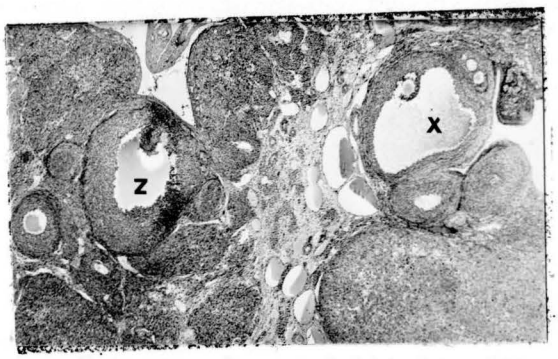

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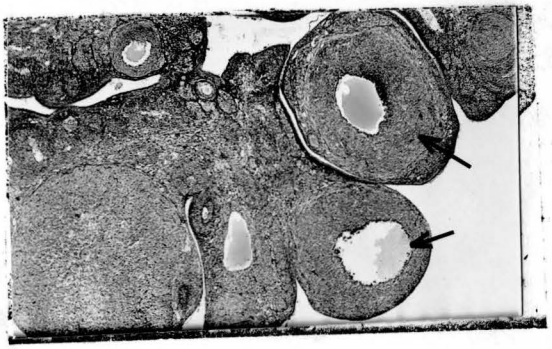


D.

FIGURE 7

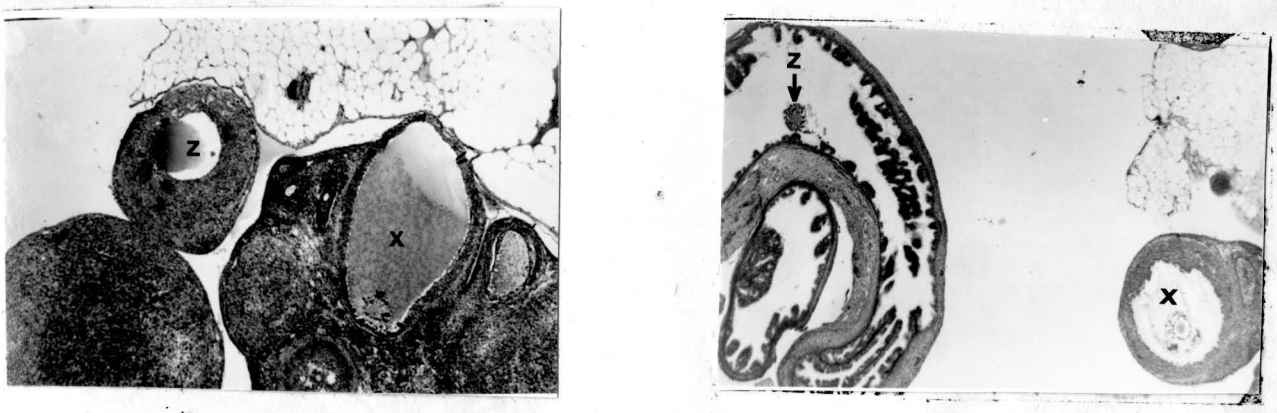
Sections of ovaries from control animals in the 2-Month Study. 80X.

A. Proestrus. Two developed follicles. Degenerating corpus luteum in lower left. Animal SD15.

B. Estrus. General type of follicle found in control estrous animals. Animal SD34.

C. Estrus. An unusually highly stimulated follicle rarely found during estrus (x). Corpus luteum with lumen (z). Animal SD3.

D. Estrus. Corpora lutea with prominent lumenal cavities. Animal SD 38.



A.

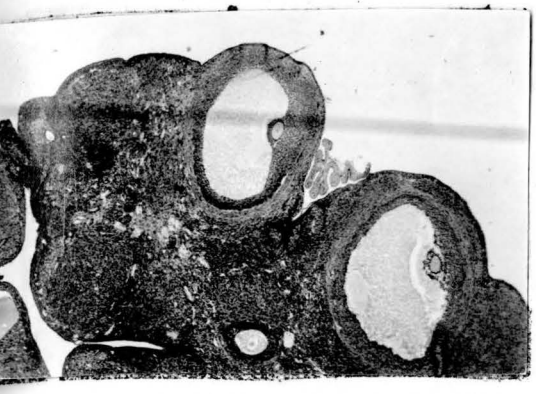
B.

FIGURE 8

Sections of ovaries from ACO-sham lesioned animals in the 2-Month Study. 80X.

A. Estrus. Large follicle with separating ovum surrounded by granulosal cells (x). Corpus luteum with large lumen (z). Animal SD40.

B. Estrus. Follicle with Ovum surrounded by granulosal cells free within follicular fluid (x). Egg within oviduct (z). Animal SE5.



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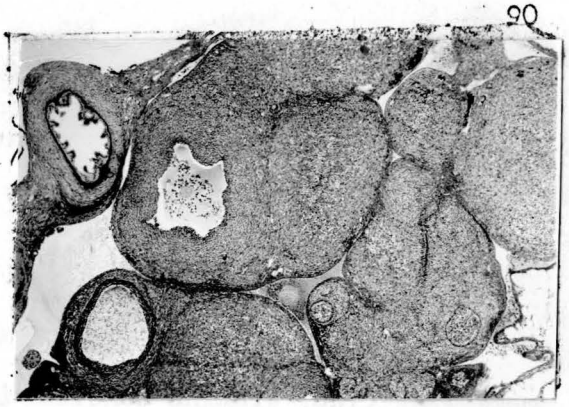

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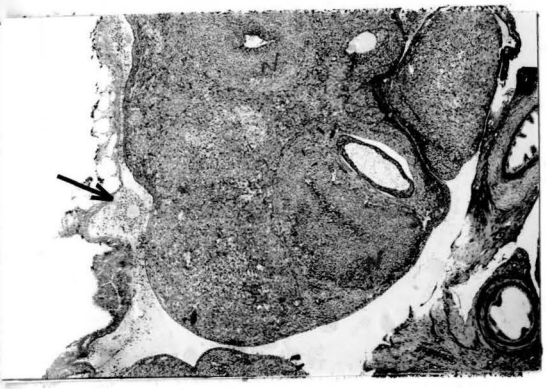
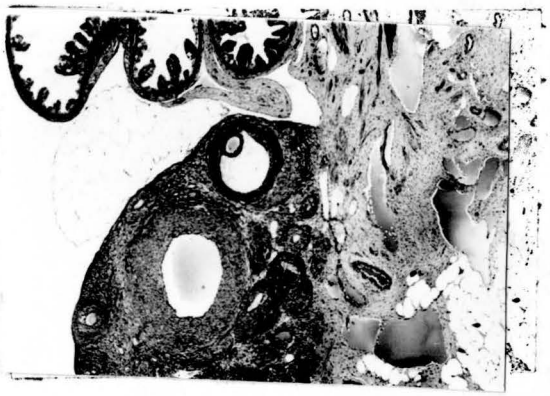


C.



D.

FIGURE 9

Sections of ovaries from ACO-lesioned animals in the 2-Month Study. 80X.

A. Estrus. Highly stimulated follicles. This animal had 3 oviductal eggs and 5 highly stimulated follicles. Animal SD59.

B. Estrus. Highly stimulated follicle; ovum surrounded by granulosal cells is separated and floating free within the follicular fluid. Animal SD32.

C. Estrus. The only egg ovulated by Animal SD32. It is still within the ovarian intracapsular space.

D. Estrus. Fresh corpus luteum with prominent lumen. Animal SD7.

follicular luteinization found in estrous ovaries of control animals. In one ACO-lesioned animal (Figure 9) ova were not found in the oviduct, but one was located between the ovary and the ovarian capsule. Not all follicles found in estrous ACO-lesioned animals were heavily luteinized, nor did all lesioned animals have these large follicles. One ACO-lesioned animals (SD7) autopsied during estrus had no follicles comparable to those found during proestrus.

These histological data, in conjunction with the difference noted in the number of ova during estrus-AM and estrus-PM in ACO-lesioned animals (Table XIV) suggests that the ovulatory process was disturbed in animals with lesions in the ACO.

3. Direct Measurement of Luteinizing Hormone (LH)

Plasma LH was not significantly different among the three time periods studied in any of the experimental groups (Table XVI), despite the fact that proestrous levels were somewhat higher than estrous levels throughout. No significant differences were noted, either, among the experimental groups when respective time periods were compared. It thus seems evident that peak release of the ovulatory surge of LH in the control was still occurring later than 3:30 PM and that two months after lesioning the ACO, AL, or "ST" 1) the low base-line LH levels were not grossly altered, and 2) the critical period for LH release had not been advanced so as to be apparent within the autopsy time period.

Pituitary LH content in control animals was found to be significantly lower on the morning of estrus than it was at proestrus, confirming the fact that an ovulatory surge of LH had been released. By estrus-PM a buildup of new pituitary LH stores seemed underway; the afternoon estrous level did not differ significantly from either the proestrous or estrous morning values (Table XVII). The same pattern was seen in pituitary LH content in the ACO-sham lesioned

TABLE XVI

PLASMA LH CONCENTRATIONS IN THE 2-MONTH STUDY ANIMALS

	PROESTRUS	ESTRUS - AM	ESTRUS - PM
CONTROL	44.3*±19.3 (8)**	18.7± 9.3 (6)	46.8±17.8 (10)
ACO-SHAM	65.7±27.1 (6)	50.6±19.1 (9)	32.6± 7.7 (12)
ACO-LESION	47.9±15.3 (11)	18.1± 7.7 (9)	21.4± 8.3 (10)
ST-SHAM	---	16.9± 7.1 (9)	---
ST-LESION	53.1±18.0 (5)	43.7±16.4 (3)	30.4±11.4 (4)
AL-SHAM	21.9±13.0 (8)	12.8± 4.4 (10)	---
AL-LESION	20.4± 8.1 (6)	11.7±11.7 (2)	---

*ng/ml (NIH-LH-S_{13})

**Mean ± SEM (Number of Animals)

TABLE XVII

PITUITARY LH CONTENT IN THE 2-MONTH STUDY ANIMALS

	PROESTRUS	ESTRUS - AM	ESTRUS - PM
CONTROL	23.1*[a] (18.8 - 28.4)† 3‡	13.3[a] (10.4 - 16.9) 3	16.2 (13.0 - 20.2) 3
ACO-SHAM	22.5[b] (18.3 - 27.5) 3	13.4[b] (10.1 - 17.9) 3	17.0 (13.5 - 21.4) 3
ACO-LESION	17.9 (15.1 - 21.1) 4	14.3 (11.2 - 18.2) 3	19.2 (15.4 - 23.8) 4
ST-LESION	15.8 (12.3 - 20.2) 3	11.7 (9.1 - 15.0) 3	15.0 (10.5 - 21.0) 3

*Weighted Mean Potency [μg(NIH-LH-S_{13})/pituitary]

†95% Confidence Limits

‡Number of Assays Combined

[ab]Potencies Significantly Different ($p<.05$)

animals. The anesthesia, surgery, and CNS tissue damage inflicted by the sham procedure apparently had not seriously disturbed the reproductive neuroendocrine network. However, pituitary LH content in the ACO-lesioned animals was not found to fluctuate between proestrus and either estrus-AM or estrus-PM. The low proestrous content most probably accounts for the absence of any significant drop between proestrus and estrus-AM. This content, however, was not significantly lower than that in the controls. Pituitary LH content in the "ST"-lesioned animals followed a pattern similar to that in the ACO-lesioned group.

C. The Four-Month Study

The data obtained from individual animals is recorded in Appendices V, VI, and VII. As in the previous studies, lesion placement was very accurate. Most lesions involved the posterior-medial part of the ACO and some of the lateral-posterior portion of the AME (Figure 2). Lesions in the internal capsule, and hippocampus were not histologically verified. Lesions directed at the DMNT and the POA-SchN were found to be appropriately placed.

Inspection of Table XVIII reveals that body, pituitary, thyroid, and adrenal weights were not altered from those of controls in either the ACO-sham or -lesioned animals. This, along with the excellent appearance of the animals, indicates general health was good. The pituitary was functioning reasonably normally as indicated by maintenance of its weight and that of the thyroids, adrenals, and ovaries. Autopsies were performed at 208-250 days of age in controls, 202-248 days in the shams, and 202-247 days in the ACO-lesioned animals. Sham animals were autopsied 116-140 days after the operative procedures. Lesioned animals were autopsied 112-132 days after placement of lesions.

1. Indirect Assessment of Circulating Ovarian Steroid Levels

a. Vaginal cyclicity

Vaginal cycles in the ACO-, DMNT-, Hipp-, and IC-lesioned animals were not obviously different from those seen in control or sham ACO-lesioned animals. The vaginal smear was only cornified during estrus and no abnormally high amounts of cornified cells were found during other stages of the cycle (Appendix II). Lesions in the preoptic area in the vicinity of the suprachiasmatic nucleus stopped the cycle and induced a state of vaginal persistent estrus (Appendix II). This demonstrates that the size of the lesion was physiologically effective.

A detailed analysis of post-lesion cyclicity was not made in this study

TABLE XVIII

BODY, PITUITARY, THYROID, AND ADRENAL WEIGHTS, AGE,

AND DAYS POSTLESION AT AUTOPSY OF THE 4-MONTH STUDY ANIMALS

	Body Weight (gm)	Pituitary Weight (mg)	Thyroid Weight (mg)	Adrenal Weight Wet (mg)	Age at Autopsy (Days)	Days Post-Operative
CONTROL	371±11 (13)†	15.7±1.0 (12)	20.0±1.5 (13)	31.1±1.6 (12)	231 (13)	----
SHAM	370±12(8)	16.3±1.2 (8)	22.0±1.7 (8)	31.7±1.4 (8)	231 (8)	123 (8)
LESION	361± 9 (19)	15.6±1.0 (19)	18.9±1.2 (19)	31.2±1.6 (19)	220 (19)	121 (19)

†Mean ± SEM (Number of Animals)

because the pre-lesion cyclic history had not been rigidly controlled. Some animals were regular four-day cyclers, some regular five-day cyclers, but most were irregular four- and five-day cyclers. The rigid criteria were imposed on the two-month study after it was apparent from this study that no obvious disturbance had occurred after the ACO-lesions were performed.

b. Uterine changes

Although uterine wet weights in all groups were lower on the afternoon of estrus than at proestrus, in no case was the difference significant (Table XIX). Uterine dry weights also did not show a significant drop at estrus-PM compared to proestrus; however, in the lesioned animals, uterine dry weight at estrus-AM was significantly lower than at proestrus ($p<.05$). No significant difference was found between the control proestrous and estrous-PM percent dry weights. The ACO-lesioned data demonstrate a significantly greater ($p<.05$) percent dry weight during estrus than at proestrus-PM.

Table XX contains summarized data on the uterine lumenal fluid content at proestrus. There was no difference in the amount of uterine lumenal fluid at proestrus among the experimental groups. No lumenal fluid was found during estrus. It seems, then, that the diestrous steroid levels inducing the accumulation of lumenal fluid (12, 251) are not seriously altered four-months after ACO lesioning, and that the postovulatory steroid changes are not markedly affected by the lesions (12, 190).

c. Pituitary and adrenal weights

The lack of a significant difference in pituitary or adrenal weights between control and ACO-lesioned animals also indicated no markedly changed (elevated or reduced) estrogen levels in the ACO-lesioned animals (106, 223).

TABLE XIX

UTERINE DATA IN THE 4-MONTH STUDY ANIMALS

	PROESTRUS	ESTRUS - AM	ESTRUS - PM
CONTROL	A. 640.0 ±45.0 (8)†	-----	493.0 ±55.6 (5)
	B. 114.2 ± 5.5 (4)	-----	112.7 ±21.8 (3)
	C. 19.7 ± 0.8 (4)	-----	20.8 ± 0.8 (3)
SHAM-ACO	A. 586.2 ±63.4 (3)	-----	578.6 ±49.7 (5)
	B. 131.0 (1)	-----	121.5 ±12.2 (4)
	C. 18.8 (1)	-----	21.5 ± 0.8 (4)
LESION-ACO	A. 633.0 ±51.7 (5)	518.5 ±30.4 (4)	550.0 ±34.9 (10)
	B. 129.1 ± 0.1 (2)	100.5 ± 5.2 (4)	108.7 ± 9.2 (8)
	C. 17.4 ± 0.4 (2)	19.4 ± 0.4 (4)	19.8 ± 0.5 (8)

†Mean ± SEM (Number of Animals)

[A]Wet weight (mg)

[B]Dry Weight (mg)

[C]Percent Dry Weight (Dry Weight/Wet Weight X 100%)

TABLE XX

UTERINE INTRALUMENAL FLUID AND NUMBER OF OVA

OVULATING THE 4-MONTH STUDY ANIMALS

	Uterine Intralumenal Fluid (mg)	Tubal Ova	
	PROESTRUS	ESTRUS - AM	ESTRUS - PM
CONTROL	530.6±103.5 (6)† (224.8-965.9)*	-----	6.6±1.0 (5)
SHAM-ACO	144.0± 43.4 (2) (100.6-187.4)*	-----	9.5±1.9 (4)
LESION-ACO	526.5±271.7 (3) (151.0-1054.4)*	7.5±1.3 (4)	6.9±0.6 (10)

†Mean ± SEM (Number of Animals)

*Range

2. Indirect Assessment of Plasma Gonadotrophin Levels

a. Ovarian weight

Significant differences in ovarian weight were not found within the cycle for any experimental group or among groups at a given stage of the cycle (Table XXI). This indicates no marked increase or decrease in circulating gonadotrophin levels was present four months after lesioning (88).

b. Ovulation

Table XX summarizes data on the number of tubal ova (unilateral) counted in estrous animals; ova were not found during any other stage of the cycle. The number of ova does not differ significantly in any of the groups. It thus seems that four months after lesioning the ACO, the plasma gonadotrophin levels needed for folliculogenesis and ovulation (88) have not been seriously altered.

c. Ovarian histology

Study of the ovarian histology reveals that the control and ACO-lesioned animals had corpora lutea with large lumen (Figure 10) during estrus.

3. Direct Assessment of Plasma and Pituitary LH

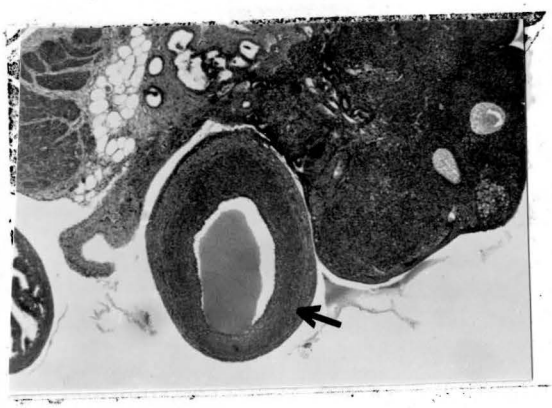
Plasma LH concentrations (Table XXII) at proestrus and estrus were not significantly different in the controls. A significant difference ($p<.01$) between proestrus-PM and estrus-PM was found in the sham ACO-lesioned animals, but the respective values were not different from those in control animals; the proestrous value however approached significance ($.05<p<.1$). Analysis of the data from ACO-lesioned animals shows statistical significance between the proestrous and estrous-PM titers ($p<.01$), while the proestrous plasma titers in the lesioned rats were significantly ($p<.01$) higher than in controls. The proestrous data of the sham and lesioned animals were not significantly different. The values during estrus-PM in both control and sham animals do not

TABLE XXI

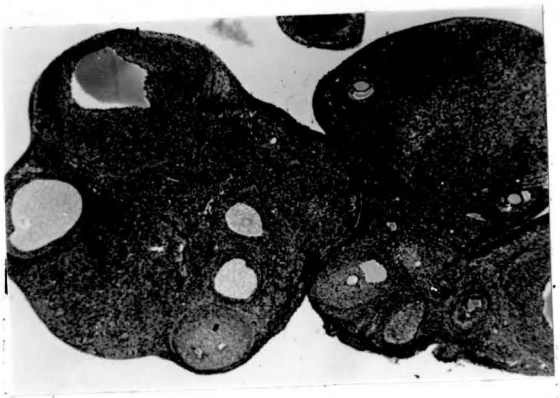
OVARIAN WEIGHT DATA (-mg) IN THE 4-MONTH STUDY ANIMALS

	PROESTRUS	ESTRUS - AM	ESTRUS - PM
CONTROL	41.2±1.5 (8)†	-----	43.4±3.5 (5)
SHAM-ACO	41.2±4.4 (3)	-----	44.2±2.3 (5)
LESION-ACO	38.9±3.2 (5)	41.5±1.1 (4)	40.9±3.3 (10)

†Mean ± SEM (Number of Animals)



A.



B.

FIGURE 10

Ovaries from the 4-Month Study. All 80X.

A. Control/Estrus. Corpus luteum with large lumen. Animal SA7.

B. ACO-lesioned/Estrus. Corpus luteum with large lumen. Animal SA27.

TABLE XXII

PLASMA LH CONCENTRATIONS‡ IN THE 4-MONTH STUDY ANIMALS

	PROESTRUS	ESTRUS - PM
CONTROL	12.8 ± 9.4^{c} (6)†	0.0±0.0 (5)
ACO-SHAM	67.2 ± 30.9^{a} (3)	0.0 ± 0.0^{a} (7)
ACO-LESION	137.0 ± 61.8^{bc} (3)	13.3 ± 7.4^{b} (9)

†Mean SEM (Number of Animals)

‡ng/ml (NIH-LH-S_{13})

$^{abc}p<.05$

necessarily indicate an absence of LH in the plasma, but rather that the amount present was at, or lower than, the minimal detectable level of the assay technique. These data offer evidence that the critical period had been advanced in these ACO-lesioned animals; a marginal effect was indicated in the sham animals.

Table XXIII summarizes pituitary LH content in control, sham, and lesioned animals. No differences were found as a function of cycle stage or among control, sham, and lesioned animals at a given cycle stage.

TABLE XXIII

PITUITARY LH POTENCY 4-MONTHS POSTLESION

	PROESTRUS		ESTRUS		METESTRUS
	AM	PM	AM	PM	PM
CONTROL		16.5* (13.7 - 20.0)† 3‡	10.1 (7.2 - 13.8) 1	15.2 (11.5 - 19.9) 3	14.2 (9.1 - 22.7) 1
ACO-SHAM	21.9 (16.2 - 29.5) 1	15.6 (12.2 - 19.9) 2	14.0 (8.7 - 22.5) 1	15.8 (12.3 - 20.3) 3	
ACO-LESION		18.7 (14.4 - 24.3) 2		14.7 (11.7 - 18.6) 4	

*Weighted Mean Potency

†95% Confidence Limits

‡Number of Assays

D. The Ovarian Compensatory Hypertrophy (OCH) Study

Table XXIV summarizes the results from this study. Appendix VIII documents the original data. If 10, 15, or 20 days were allowed to intervene between removal of the left and right ovaries, the percent difference in weight (% OCH) was significantly greater than that observed if both ovaries were removed on the same day. Although there were no significant differences among the 10-, 15-, and 20-day OCH responses, the difference between the 10- and 20-day OCH response approached significance ($.05<p<.1$). The experiments were carried out with the 10-day OCH response because the response was still in a dynamic state (Control data, 78), and it was felt that an evaluation in the dynamic state would yield more information than if the OCH response were measured after an equilibrium had been established. This seems justified when the following general representation of a dynamic system is considered:

$$G(t) = A_0 g_0 + A_1 \frac{d'}{dt'} g_1 + A_2 \frac{d''}{dt''} g_2 + \cdots$$

$G(t)$ = An arbitrary time-dependent phenomenon

$A_0, A_1, \cdots$ = Arbitrary coefficients

$g_0, g_1, \cdots$ = Arbitrary time response functions which $G(t)$ is dependent on

$\frac{d'}{dt'}, \frac{d''}{dt''}, \cdots$ = time derivatives

At equilibrium, all time derivatives are zero and $G(t) = A_0 g_0$. If $G(t)$ is now designated the OCH response, only the steady-state term ($A_0 g_0$) would be evaluated for normalcy, were the rats autopsied during a static condition. However, if the evaluation is made during the response period, (an unspecified number of) the time derivative terms would also be included in the evaluation, possibly giving a better indication of whether the system is disturbed but no hint as to the functional origin of the disturbance.

Placement of lesions in the ACO were excellent with one exception (Appendix VIII; Misplaced Lesions). In this animal, the left ACO was destroyed

TABLE XXIV

OVARIAN COMPENSATORY HYPERTROPHY STUDY

	DAYS BETWEEN REMOVAL OF THE FIRST & SECOND OVARIES	% OCH MEAN ± SEM (Number of Animals)	SIGNIFICANCE
CONTROL	0	3.55±3.70 (27)	abcdef
	10	35.68±4.71 (18)	agh
	15	45.33±10.21(9)	b
	20	51.54±6.98 (10)	c
ST-SHAM	10	41.35±8.18 (9)	di
ACO-SHAM	10	34.63±4.14 (10)	ej
ST-LESION	10	2.38±4.54 (6)	gik
ACO-LESION	10	4.00±5.97 (8)	hjl
CEREBRAL CORTEX-LESION	10	33.06±9.59 (6)	fkl

a through h = $p<.01$

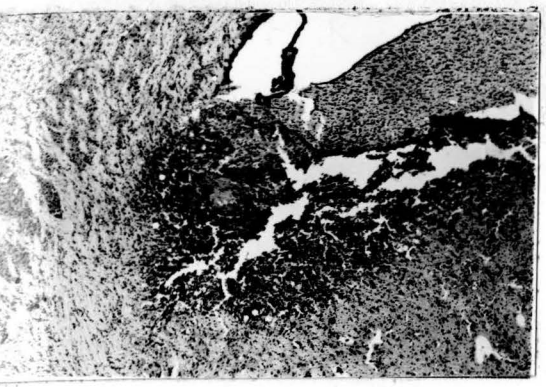
i and j = $p<.02$

k and l = $p<.01$

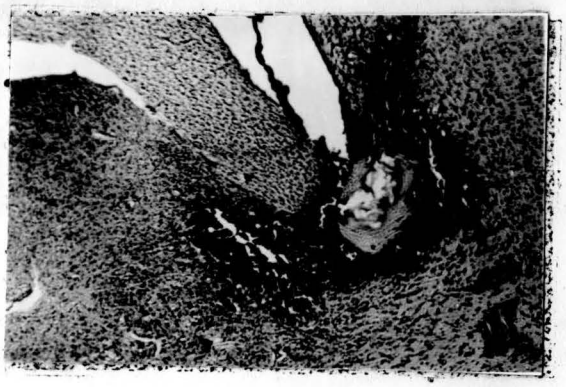
but the right ACO was left intact; the lesion was very small (0.2-0.5 mm) and in the cortex surrounding the medial nucleus. This animal was not used in evaluation of the OCH response in ACO-lesioned animals.

More precise placement of ST lesions was achieved in this study than in the 2-month study because of a 0.5 mm more dorsal placement. In three cases (SD54, SD124, and SG3) the ST was extensively damaged bilaterally, in three other cases (SG1, SG2, and SG20) the ST was badly damaged on one side while partial damage was inflicted on the other side (Figure 11). The ST lesions in two animals (SD92 and SI58) were judged inadequate. While the ST on the left side was lesioned, that on the right incurred no obvious destruction in animal SD92 (Figure 12). The lesions in animal SI58 were both too dorsal and neither ST was damaged. The ST appears to be a high resistance path and thus less readily destroyed by electrolytic lesioning than the surrounding tissue. This was especially dramatized with placements very close to, and on the border of, the ST; the spread of tissue damage into the ST was not as great as that in other directions away from the ST. Even with lesions directly in the ST, less extensive damage was done to the adjacent ST tissue than to adjacent non-ST tissue (Figure 12).

As can be seen in Table XXIV, lesions of the ACO prevented the 10-day OCH response from occurring. Damage to the ST also blocked the 10-day OCH response, whereas, lesions in the cerebral cortex did not. In that sham procedures (ST and ACO) did not interfere with the OCH response, trauma due to anesthesia, surgery, or brain-damage incurred by lowering the electrodes were apparently negligible. Since neither lesions of the cerebral cortex nor the misplaced lesions had any significant influence over the OCH response, possible diffusion of iron deposited at the lesion site into the general and/or cranial circulation (Figure 13) also probably had little if any influence over the OCH



A.



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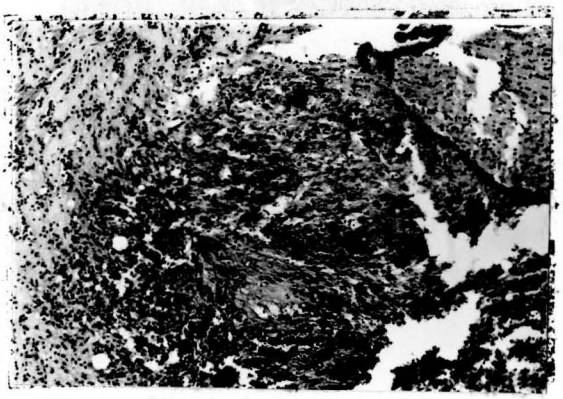

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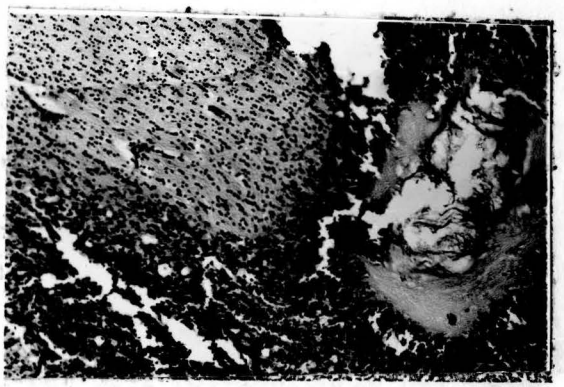
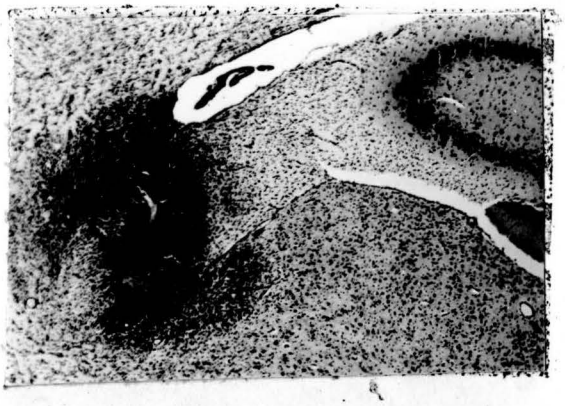


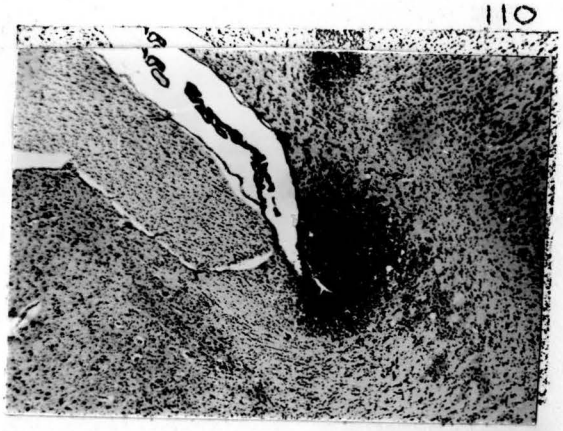
D.

FIGURE 11

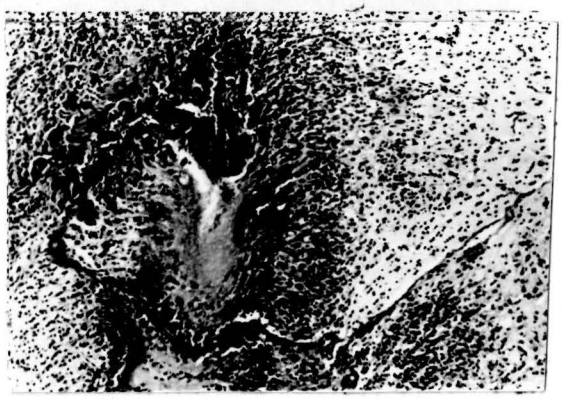
Photomicrographs of stria terminalis lesions in rat SG20 (ST-Lesion/10 Day OCH). The left ST (A and B) was extensively damaged; the right ST (C and D) had incurred damage but not as extensively. (A and C are 80X; B and D are 200X).



A.



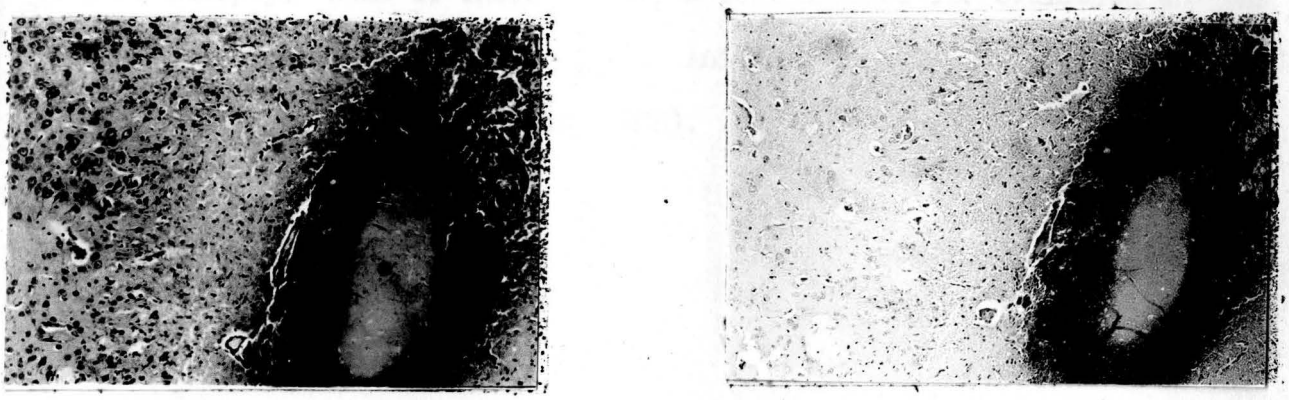
C.



B.

FIGURE 12

Photomicrographs of stria terminalis lesions in rat SD92 (ST-lesion/10 Day OCH). The left stria terminalis was significantly damaged (A and B) while the right (C) was not. (A and C are 80X, B is 200X).



A. B.

FIGURE 13

ACO Lesion with Iron Stain

ACO lesion in rat LG2; both photomicrographs are 200X. A. section stained with cresyl echt violet. Several prominent nerve cells are readily observed on the far left; a region devoid of the prominent nerve cells is found before the major lesion site as the photomicrograph is inspected from left to right. B. section stained by Gomori's Iron Reaction using nuclear fast red as the counter stain. Note that the extent of iron deposition does not extend beyond the boundaries of the major lesion site into the region devoid of large nerve cells.

response.

Since the right ovaries were removed ten days after the lesioning-unilateral ovariectomy procedure, animals were in various stages of the estrous cycle at autopsy. Some of those in estrus were checked for ovulation and the number of eggs counted (Appendix VIII). Animal SD119 had 13 eggs at autopsy (Appendix VIII: ACO-Lesion 10-days OCH).

CHAPTER V

DISCUSSION AND CONCLUSIONS

A. General Considerations

Deposition of iron (Figure 13) was found in all lesion sites, whether in the acute 3-cycle study or the chronic 4-month study. Since electrolytic deposition of iron in the brain has been shown capable of stimulating gonadotrophin secretion (89, 91), the possibility that the lesion sites may have acted as stimulative foci must be considered. It is felt the lesions in this series of studies were not stimulative because: 1) the lesion effects were seen for more than a week after placement (no studies were found in the literature showing effects of electrolytic stimulation lasting more than several hours), 2) lesions of the ST had the same effects as lesions in the ACO region (OCH study), 3) the region of iron deposition was infiltrated by connective tissue and a border around the deposition was heavily infiltrated by glial cells and very few if any nerve cells (Figure 13), and 4) a functional difference between electrolytic lesions and electrical stimulations in the amygdala has been reported (22, 76).). Thus it is felt that the ACO lesions induce a reduction of ACO neural efferent traffic, impairing the information-processing potential and/or efficiency, and subsequently removing the functional role which this region may play in physiological systems.

The mild effects of sham lesions may result from destruction of CNS tissue as the electrodes were lowered. Lawton and Sawyer (175) have demonstrated that inadvertent destruction of ST tissue by the electrodes can induce changes in the sham animals similar to those in ACO-lesioned rats. In the

present experiments 26 Gauge hypodermic tubing was used in an attempt to eliminate the interference previously found (175) using 24 Gauge tubing. Even smaller gauge tubing may be preferable when working in the amygdalar region since some effects due to sham lesioning seem apparent, although not nearly as marked as when 24 Gauge tubing was used (175). Although sham effects were apparent in the 3-cycle study, they were absent in the acute OCH-study. This discrepancy may reflect differing sensitivities of the FSH and LH systems to the destruction of nonspecific and/or specific CNS tissue in the path of the electrode.

The specific location of CNS lesions was important as demonstrated by the profound effects of POA-SchN lesions on the estrous cycle which were not seen following ablation of the other sites (Appendix II). The difference in the OCH response following lesioning of the ACO and cerebral cortex also demonstrate that "nonspecific" lesions do not necessarily have effects on the measured physiologic end point.

Although thyroid weights in lesioned animals were not significantly altered from control in any of the three cyclic studies, data from the 2-month study suggests that the ACO and AL have opposing influences (inhibitory and stimulatory, respectively) on the maintenance of thyroid weights. Thyroid weight has been reported to be reduced following medial amygdaloid lesions in the male deermouse (75); however, effects beyond 16 days post-lesion were not studied. It is thus possible that at 2-months post-lesion, the beginning or end of a phase in which the ACO lesioned rats have higher weights and the AL-lesioned animals have lower weights than controls for some period is present. At 2-months post-lesion enough of a drift was present to see a significant weight difference between the ACO- and AL-lesioned rats. These findings strengthen

the notion that the various changes observed following lesioning in the present series of experiments were dependent upon ablation of specific CNS sites rather than upon ion deposition since the ACO and AL lesions involved regions of the brain barely one millimeter apart.

The suggestion that lesions of the corticomedial region of the amygdala may elevate thyroid weight seems in direct opposition to the results of Eleftheriou and Zolovick (75). This difference, however, may be explained by species differences, sex differences, duration of experiment, and/or specificity of lesion localization since lesions in the male deermouse were apparently confined to the medial nucleus while very little damage was done to the medial nucleus in the present studies.

B. Circulating Estrogen and Progesterone Titers

The increase in uterine weights and tissue fluid, and the cornification of the vaginal epithelium normally observed during proestrus result from an increase in ovarian steroid concentrations in the blood during diestrus (12, 13, 174, 248, 251). The accumulation of uterine lumenal fluid during proestrus depends not only on high estrogen levels but on relatively low circulating progesterone levels as well (12, 251); if progesterone titers are too high (<40 ugm), uterine fluid will not accumulate in the presence of estrogen concentrations that would otherwise be effective (12). In all three cyclic studies, the lesioned animals demonstrated normal proestrous uterine weights, tissue and lumenal fluid values, and vaginal cornification. It thus appears that ovarian steroid titers at diestrus were not grossly different from normal values. Furthermore, a normal time course for reduction of estrogen levels and increase of progesterone levels was indicated by the decreasing uterine weights and tissue fluid during estrus and metestrus (13, 248, 251, 274), the loss of the uterine lumenal fluid (12, 251), the appearance of a lukocytic vaginal smear

by metestrus (88), and the normal duration of the estrous cycles (88). If progesterone titers had been deficient following the ovulatory surge of gonadotrophins, one would have expected to find lumenal fluid during estrus and possibly even metestrus (12), as well as a prolongation of the estrous vaginal smear (88). In as much as increases in pituitary (106) and adrenal (223) weights can reflect increased plasma estrogen levels, the failure to not note any consistent significant changes post-lesioning in the present experiments further supports the absence of any major alterations in circulating estrogen levels.

The data from these studies, therefore, strongly indicate that plasma estrogen and progesterone concentrations throughout the cycle had not been seriously altered by ACO- or AL-lesions. Although elevated numbers of cornified cells have been reported in vaginal smears following amygdaloid electrolytic lesions (45), the lack of details in that abstract makes it difficult to reconcile this difference.

C. Gonadotrophin Levels

Maintenance of ovarian weight in all experiments (Tables III, XV, and XXI) indicates there was no dramatic increase or decrease in basal gonadotrophin secretion due to the ACO- or AL- lesions. This is in contrast to a recent paper (23) in which Bekhtereva reports increased ovarian weights following lesions of the medial and lateral amygdala; however, since many animals in that study were apparently pseudopregnant when autopsied, the larger corpora lutea of pseudopregnancy (30) could account for the elevated ovarian weights. The comparable number of ova found in control and experimental animals in all studies also indicates that adequate FSH and LH titers were present to stimulate folliculogenesis (98, 190), estrogen secretion (98, 190), and ovulation (88). Direct assessment of LH secretion also failed to indicate any consistent eleva-

tion of LH secretion throughout the cycle either acutely (2-3 weeks) or chronically (2 or 4 months) following ACO lesioning, in contrast to other studies indicating marked elevations of plasma LH two (23, 74) to three weeks (175) after lesioning. In that these results were obtained from pseudopregnant (23, 74) and ovariectomized rats (175) the findings cannot be expected to closely correlate with the results of the present studies performed on cyclic animals, in which steroidal feedback influences upon gonadotrophin secretion are quite different and both basal and cyclic secretion of gonadotrophins and steroids are operative. The lack of any consistently prominent elevation in plasma LH in cyclic animals of this study was probably due to feedback influences of the normal or near-normal ovarian steroid levels; such feedbacks were altered (23, 74) or inoperative (175) in the other studies. A careful assessment of gonadotrophin secretion by indirect and direct means at specific times during the various stages of the estrous cycle in control and lesioned animals, however, did reveal several definite indications of subtle changes, as discussed below.

Histological studies of the ovaries revealed retarded luteinization in most of the ACO-lesioned animals. The signs of reduced luteinization were apparent at metestrus - even after apparently mildly elevated plasma LH during estrus-PM in the 3-cycle study. These data, along with the retarded ovulation seen in ACO-lesioned animals in the 2-month study, although possibly being suggestive of hyposecretion of LH at proestrus in ACO-lesioned animals, is not so interpreted. The fact that luteinization has started, uterine lumenal fluid is absent at estrus, the estrogen plasma levels are dropping by estrus-AM, and a normal quota of ova are released, indicates that high plasma LH levels most likely occurred (12, 13, 98, 190, 209, 274). More importantly, the significantly lower pituitary LH content at estrus-AM than at proestrus in the

3-cycle study although indirect, indicates the occurrence of an LH ovulatory discharge similar to that of controls elevating plasma LH between proestrus-PM and estrus-AM. It is thus curious why full luteinization did not occur.

The retardation may be due to follicular refractoriness to the ovulatory levels of gonadotrophins; abnormal maturation of the follicles may occur. This may also explain the discovery of large non-ovulated follicles in the ACO-lesioned estrous animals. The abnormal maturation may have its origin in the disturbed FSH-ovarian steroid feedback mechanism - i.e., FSH titers may be marginally sufficient for folliculogenesis (98, 190). Even with FSH reduced, adequate LH and a minimal amount of FSH could allow follicular maturation (98, 190), but abnormal in some biochemical way as a result of low FSH, thus resulting in ruptured follicles which do not completely luteinize by metestrus. Although there is no direct evidence, low levels of prolactin may be implicated since it is apparently luteotrophic in rats (88).

An additional subtlety induced by the ACO-lesions was the accelerated reaccumulation of pituitary LH found in the 3-cycle study. This may reflect an increased release of LH-RF (23). This also extends the inhibitory action of the posterior basal amygdala over gonadotrophin secretion seen in specialized non-cyclic rats (22, 23, 74, 76, 77, 175) to the cyclic rat.

Since the estrous-PM pituitary LH content in controls of the 2-month study was not significantly different from that in lesioned animals but was significantly higher ($p<.02$) than that in the estrous-PM controls in the 3-cycle study controls (Table XXV), aging of the animals alone may induce an increased storage rate of pituitary LH similar to that in younger rats with ACO lesions. The 4-month study estrous-PM pituitary content, however, was not significantly greater ($.05<p<.1$) than that in the 3-cycle study. (Table XXV). This is in agreement with Labhsetwar's data (171) indicating

TABLE XXV

COMPARISON OF ACO-LESIONS ON ESTRUS-PM PITUITARY LH CONTENT

AMONG THE THREE CYCLIC STUDIES

	3-CYCLE STUDY	2-MONTH STUDY	4-MONTH STUDY
CONTROL	9.5*[ab] (7.5 - 12.5)† 3‡	16.2[b] (13.0 - 20.2) 3	15.2 (11.5 - 19.9) 3
ACO-SHAM	13.4 (10.8 - 16.7) 3	17.0 (13.5 - 21.4) 3	15.8 (12.3 - 20.3) 3
ACO-LESION	17.8[a] (14.2 - 22.3) 3	19.2 (15.4 - 23.8) 4	14.7 (11.7 - 18.6) 4

*Weighted Mean Potency

†95% Confidence Limits

‡Number of Assays

[a]Potencies Significantly Different ($p<.01$)

[b]Potencies Significantly Different ($p<.02$)

an increased pituitary LH storage in older adult female rats than younger ones. ACO-lesioning, however, did not augment the "age-dependent" rise in pituitary LH, and did not elevate the preovulatory content at proestrus in the younger animals. The ACO may have spontaneously lost the specific physiologic effect of reducing the rate of accumulation of pituitary LH it had in the younger rats by the time the older rats were autopsied, thus leaving the ACO-lesions without any relative effect on pituitary storage of LH. Alternatively, the neuroendocrine network regulating LH may have a limit as to how fast and high it can induce pituitary accumulation of LH; thus, regardless of whatever other factors are responsible for the noted age effects, the ACO-lesion is functionally ineffective in altering pituitary LH storage because some other effect was driving the network at a maximum rate of pituitary LH reaccumulation.

D. Functional Properties of the Reproductive Neuroendocrine Network

1. Circadian Elevation of Plasma LH

Evidence for a period of increased LH release in intact cyclic female rats daily during the estrous cycle has recently been presented (116). The elevation in plasma LH levels generally corresponds in time to the afternoon elevation of plasma LH found in proestrous rats and in rats bilaterally ovariectomized for five months (176), although it is less marked. This circadian LH release rhythm was not obvious in the control animals of the 3-cycle study, but its presence was suggested in the sham and lesioned animals by the elevated estrous-PM plasma LH measurements. These latter two groups had estrous-PM plasma LH concentrations which were identical to one another, but were larger than their respective estrous-AM concentrations as well as being larger than the control estrous-PM titers. However, in spite of this consistent trend, only the sham group had estrous-PM LH levels which demonstrated a significant elevation by being statistically greater than the estrous-PM

[illegible]

control values. The slight circadian rise in plasma LH during the afternoon in intact animals (116) therefore appeared augmented in ACO-lesioned and sham-lesioned animals, suggesting that the circadian stimulus may have been under less of a restraint than normal. The loss of some neural inhibitory influence was also indicated by the more rapid accumulation of pituitary LH after lesioning the ACO in the 3-cycle study. This more rapid accumulation of pituitary LH may reflect a general elevation of LH-RF secretion, inasmuch as a reduced hypothalamic LH-RF has been reported concurrent with increased plasma and pituitary LH in amygdaloid lesioned rats (23).

The significant elevation in proestrous plasma LH concentration in the ACO-lesioned rats of the 4-month study over control and estrous-PM lesion values may indicate the critical period has been advanced; although, the proestrous titers in 4-month ACO-lesioned animals were not significantly greater than those of either the 3-cycle study($p>.1$) or those in the 2-month study ($.05<p<.1$). This advancement of the critical period in the 4-month ACO-lesioned animals may reflect in a more dramatic way a heightened LH secretory potential in ACO-lesioned animals indicated in the 3-cycle study.

The reduced restraint over LH-RF may be in the form of either 1) the loss of a tonic inhibition, or 2) the loss of a circadian depressor effect over LH-RF secretion by the corticomedial amygdala which may have an inhibitive effect during the afternoon. This latter possibility is suggested by the recent work of Terasawa et al. (268) indicating cyclic activity in the amygdala associated with the estrous cycle and ovarian steroids.

2. Ovarian Steroid Feedback on FSH Secretion

If indeed the OCH response is a reflection of elevated plasma FSH (24) induced by the reduction of ovarian steroids (24, 142), the neuroendocrine network regulating FSH secretion has been seriously impaired by ACO lesions.

The network has either become so sluggish that more than ten days were required for augmented FSH release and development of ovarian hypertrophy, or the system had become more sensitive to ovarian steroid feedbacks and a new lower level of ovarian steroids was able to maintain plasma FSH at normal or near-normal concentrations.

The absence of the OCH response in POA-SchN-lesioned animals apparently reflects an abnormality in the steroidal feedback mechanism regulating FSH. This is inferred from two experimental facts. Administration of ovarian steroids after unilateral ovariectomy in non-lesioned animals not only impairs the hypertrophy of the remaining ovary (24, 142) but prevents the postcastration rise in FSH (24); thus compensatory hypertrophy is most likely a response to lowered ovarian steroid levels immediately following unilateral ovariectomy. Secondly, the abnormality is also indicated by demonstrations that while exogenous estrogen can diminish phenomena associated with FSH in non-lesioned animals, it cannot do so in POA-SchN-lesioned animals (104, 266). The lack of the 10 day-OCH response in ACO-lesioned rats is thus likely indicative of an impairment in the neuroendocrine network regulating FSH. Lesions of the ACO region may selectively destroy components involved directly or indirectly in the steroid feedback regulation of FSH. It is also possible that lesions of the ACO did not influence the ovarian feedback loop per se, but rather altered the characteristics of the internal feedback of FSH on its own secretion. That such a possibility is real can be interpreted from a recent paper by Lawton and Sawyer (175). Although the paper dealt with LH, the implication may be true of other gonadotrophins also. Having shown that ovariectomized rats with ACO lesions had plasma LH concentrations above those of non-lesioned controls, it is possible that the ACO is involved in the mediation of LH negative feedback on itself. Thus, the possibility of both "subsystems" (steroid and

gonadotrophic) being altered by ACO-lesions is possible, as well as a disturbance of a "purely neural" origin.

Since POA lesions inhibiting the OCH response also induce persistent estrus (57, 106), such lesions may either modify components in common to both 1) the ovulatory system (20, 64, 65, 66, 127, 145, 273) and 2) the FSH ovarian steroid feedback system (57, 106), or may modify several components which are independently associated with one or the other neuroendocrine networks. Although the FSH regulating system was apparently seriously interfered with after ACO lesions as well, the neuroendocrine network associated with LH regulation, functioned very close to normal if not enhanced. These data offer evidence for a diametric functional separation of these systems in ACO lesioned female rats and support the possibility that the neuroendocrine network maintaining the estrous cycle may be composed of several relatively independent "subsystems" dissectable at the CNS level.

This defect in FSH regulation was also found after ST-lesions, indicating that a part, if not all, of the informational link between the corticomedial amygdala and the hypothalamus involved in FSH regulation utilizes the ST pathway.

E. Summary

Since cyclicity and ovulation proceed in animals with an isolated POA-hypothalamic-pituitary unit (168), the maintenance of relatively normal cyclicity following destruction of the ACO is not too surprising. The ACO does appear to be exerting a net inhibitory influence over the functioning of the hypothalamic-pituitary axis as also indicated by other studies (22, 23, 74, 76, 77, 175). The present experiments extend the functional suppression by the ACO to the cyclic rat. Many of the data seem consistent with the following scheme. The POA-hypothalamic-pituitary unit has the functional capacity to cycle (168) and the circadian "ovulatory" signal (87, 88) is functionally a part of the unit. The ACO normally suppresses both the basal secretion of LH-RF and the effectiveness of the circadian release of a surge LH-RF. This may be accomplished by 1) a single direct inhibition of the hypophysiotrophic area's control over basal or tonic secretion of LH, 2) a single direct inhibition of the circadian signal, or 3) both of these by multiple inhibitory influences. The first two of these may roughly correspond to separate influences on the two neural levels theorized to be involved in the control of LH secretion (18). If, indeed, the circadian stimulus of pituitary LH secretion is augmented, as it seems to be, the ACO may be involved with the development of the favorable set of conditions leading up to the discharge of an ovulatory surge of gonadotrophins (18, 120). The important role played by hormone levels is emphasized since apparently even an increased neural stimulation of the LH release mechanism cannot advance the ovulatory discharge of LH, even though the pituitary contains a quota essentially identical to that of proestrus by metestrus in the 3-cycle study. In older animals, however, neural influences may play a proportionately greater role in lesioned animals as evidenced by the advanced critical period in ACO-lesioned animals four

months after lesioning. Some aspects of the effectiveness of the intact corticomedial amygdala of the normal rat may dwindle with age or be overridden by other influences as seen from the similarity of elevated estrous pituitary LH content between "old" control and "young" ACO-lesioned rats. The FSH regulatory mechanism is also disturbed by the ACO-lesions, but rather than being more efficient as is the LH system it is suppressed and functioning below capacity.

F. Conclusions

The following points should be emphasized:

A. Secretion of gonadotrophins, estrogen, and progesterone is not grossly disturbed by ablation of corticomedial or lateral portions of the amygdala as evidenced by cyclicity, organ weights, and LH bioassay.

B. Subtle changes occur in the regulation of gonadotrophins in ACO-lesioned rats:

1. Gonadotrophin release at proestrus in the lesioned animals is sufficient to induce ovulation but full luteinization does not occur.

2. Within the first month after lesioning of the ACO, pituitary storage of LH is more rapid.

3. The circadian release of LH appears amplified.

C. The ACO is implicated in the more rapid pituitary LH storage associated with the aging of the animals.

D. The ACO is essential for the regulation of FSH secretion.

E. The estrous cycle can be maintained despite dysfunction in the FSH neuroendocrine regulatory network.

F. The ACO is not critically involved in determining the cycle length.

APPENDIX I: INDIVIDUAL CYCLE HISTORIES FROM THE 2-MONTH STUDY

DAYS POSTLESION

ANIMAL NO.

CONTROL

ANIMAL NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
SD3			X				X				X				X				X				X				X			
SD4			X				X				X				X				X				X				X			
SD15			X				X				X				X				X				X					X		
SD17			X				X				X				X				X				X				X			
SD23			X				X				X				X				X					X				X		
SD28			X				X				X				X				X					X				X		
SD34			X				X				X				X				X				X				X			
SD36			X				X				X				X				X				X				X			
SD38			X				X				X					X					X					X				
SD48			X				X				X				X				X				X				X			
SD55			X				X				X				X				X				X				X			
SD57			X				X				X				X				X				X				X			
SD60				X				X				X				X				X				X				X		
SD71			X				X				X				X				X				X				X			
SD74			X				X				X				X				X				X				X			
SD76			X				X				X				X				X				X				X			
SD79				X				X				X				X				X				X				X		
SD82			X				X				X				X				X					X				X		
SD88			X				X				X				X				X				X				X			
SD94			X				X				X				X				X				X				X			
SD114			X				X				X				X				X				X				X			
LG38			X				X				X				X				X			X					X			
SE1				X				X				X				X				X				X				X		
SE7			X				X				X				X					X				X				X		
SE15			X				X				X				X				X					X						X
SE22			X				X				X				X				X				X				X			
SE34			X				X				X				X				X				X				X			
SE41			X				X	X				X	X				X		X	X				X				X	X	
SE53			X				X	X				X	X				X	X				X	X					X		

ANIMAL NO.	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
SD3	X				X				X				X				X				X				X				X |	
SD4	X				X				X				X				X				X				X				X |	
SD15		X					X					X				X					X					X				|
SD17	X				X				X				X				X				X				X			|		
SD23		X				X				X				X				X				X				X |				
SD28			X					X					X					X					X					X		
SD34	X				X				X				X				X				X				X				X |	
SD36	X				X				X				X				X				X				X				X |	
SD38	X				X					X					X								X						X |	
SD48	X				X				X				X				X				X				X			|		
SD55	X				X				X					X					X					X					X |	
SD57	X				X				X				X				X				X				X			|		
SD60		X				X				X				X				X				X				X |				
SD71	X				X				X					X				X				X				X |				
SD74	X				X				X				X						X					X				X		
SD76	X				X				X				X				X				X				X			|		
SD79		X					X					X					X					X					X			
SD82	X											X				X				X				X				X |		
SD88	X				X				X				X				X				X				X					
SD94	X				X				X				X				X				X				X				X	
SD114	X				X				X				X				X				X				X				X |	
LG38	X					X				X				X				X				X				X				
SE1		X				X				X				X				X				X				X				
SE7		X				X				X				X															X	
SE15				X					X					X					X					X				|		
SE22	X				X				X				X				X				X				X			|		
SE34	X				X				X				X				X	X				X	X					X		
SE41				X				X	X				X				X	X				X	X				X	X |		
SE53		X	X				X	X													X				X	X				

X = Cornified Smear

Blank Space = Noncornified Smear

– = Smear Not Taken

| = Day of Autopsy

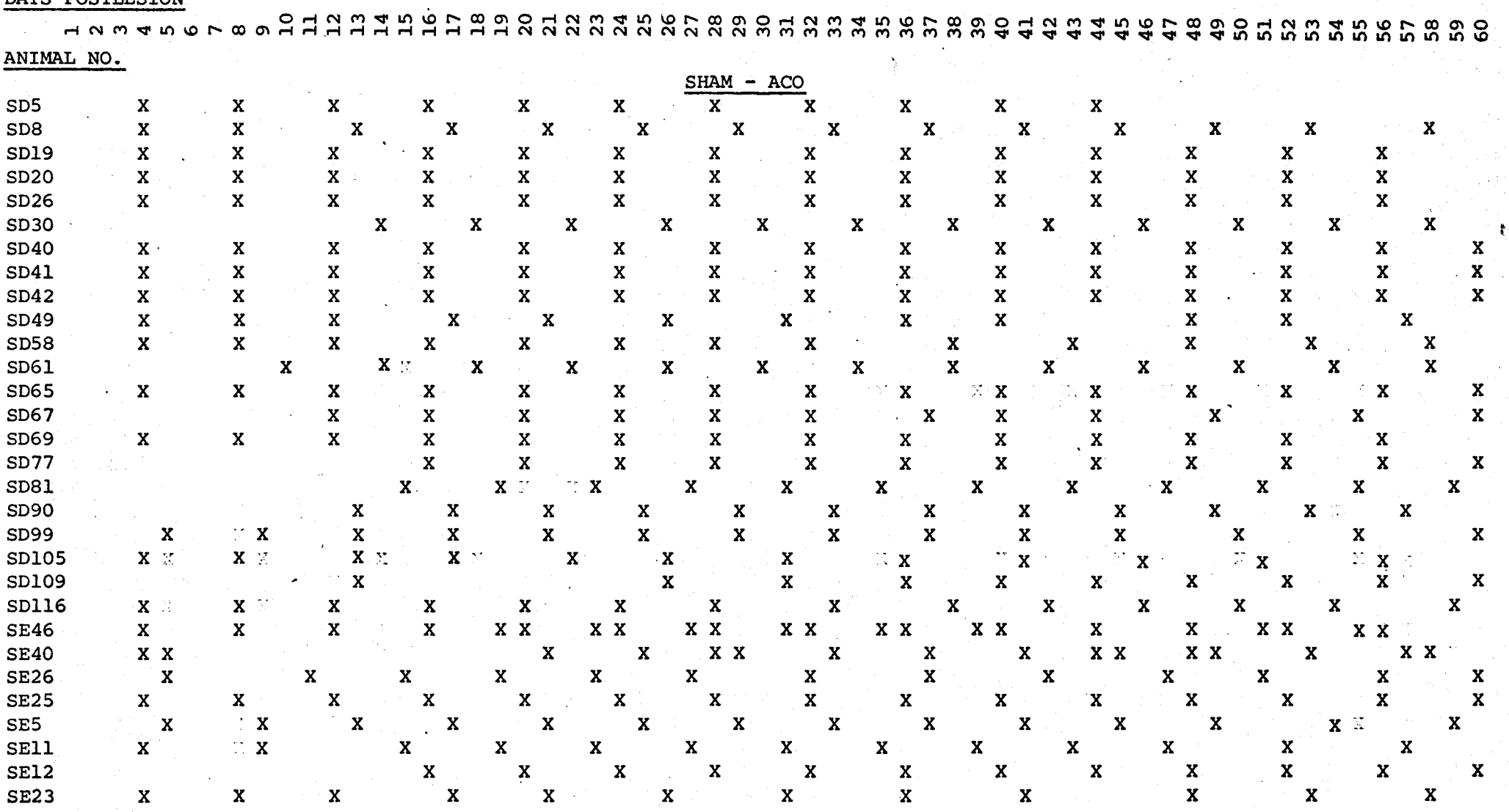
APPENDIX I: INDIVIDUAL CYCLE HISTORIES FROM THE 2-MONTH STUDY (continued)

DAYS POSTLESION

ANIMAL NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
SHAM - ST																																																															
SD13					X				X					X				X					X	X				X					X					X					X					X					X					X		
SD14												X				X				X					X				X				X				X				X				X				X					X					X
SD44				X				X				X				X				X					X				X				X				X				X				X				X					X					X
SD84				X				X				X				X				X					X				X				X				X				X				X				X					X					X
SD104				X				X				X				X				X					X				X				X				X				X				X				X					X					X
SD107				X					X					X					X					x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x					X			
SD108													X				X				X				X				X				X				X				X				X				X					X					
SD117				X				X				X				X				X					X				X				X				X				X				X				X					X					
SE17					X												X				X				X				X				X				X				X				X				X					X					
SE44				X	X				X				X				X				X					X				X	X					X	X					X	X					X	X					X	X				
LESION - ST																																																														
SD12													X				X				X				X				X				X				X				X				X				X					X					
SD21				X				X				X				X				X					X				X				X				X				X				X				X					X					
SD27												X				X				X					X				X				X				X				X				X				X					X					
SD33				X					X				X				X				X				X				X				X				X				X				X				X					X					
SD35														X				X				X				X				X				X				X				X				X				X					X				X	
SD46													X				X				X				X				X				X				X				X				X				X					X					
SD56					X				X				X				X				X				X				X				X				X				X				X				X					X					
SD66					X				X				X				X				X				X				X				X				X				X				X				X					X					
SD73														X				X				X				X				X				X				X				X				X				X					X					
SD78													X				X				X				X				X				X				X				X				X				X					X					
SD83					X				X				X				X				X				X				X				X				X				X				X				X					X					
SD96													X				X				X				X				X				X				X				X				X				X					X					
SD97													X				X				X				X				X				X				X				X				X				X					X					
SD100				X				X				X				X				X					X				X				X				X				X				X				X					X					X
SD115				X					X						X					X					X								X				X				X								X					X					

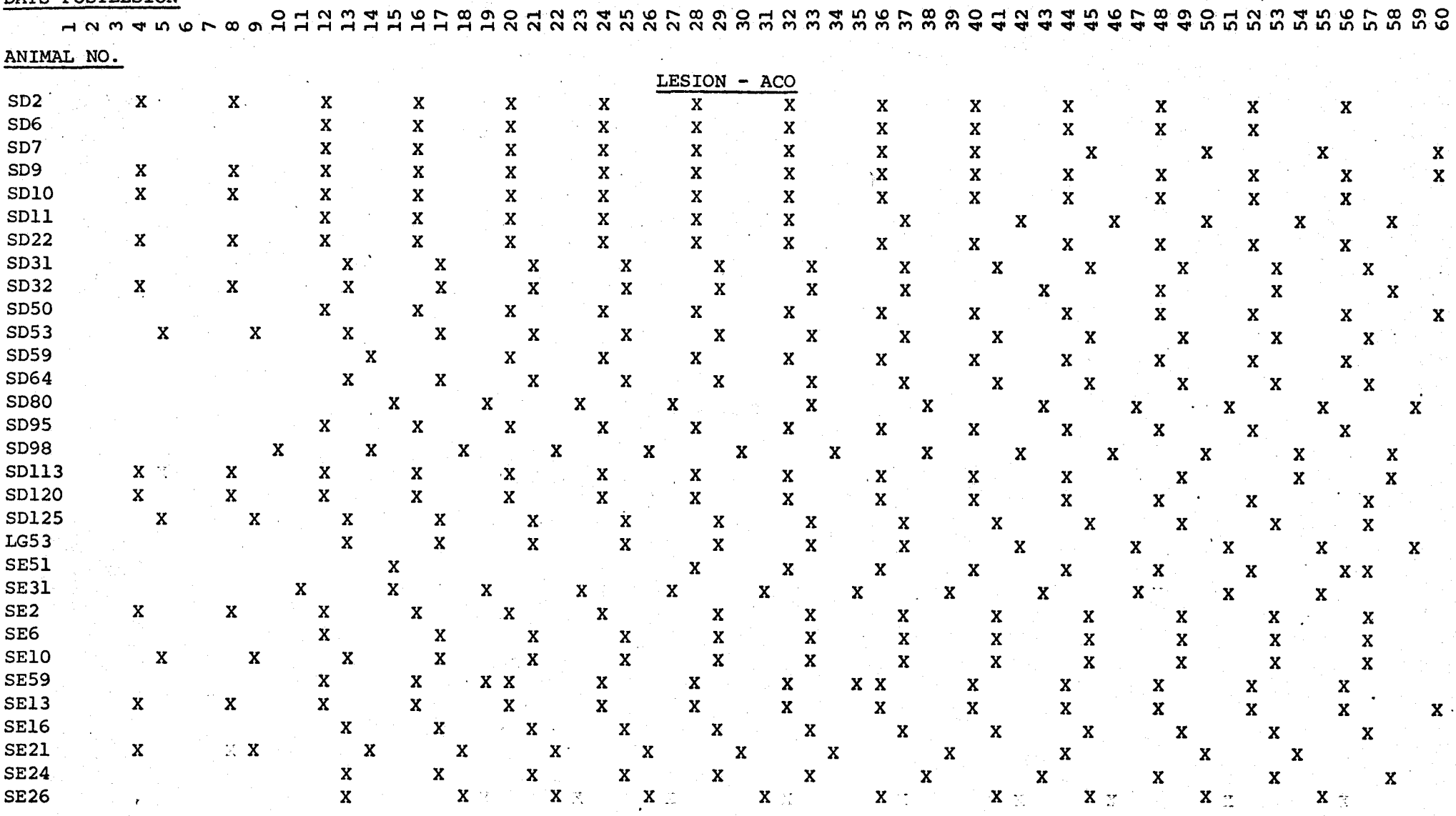
Legend: Same as APPENDIX I, page 126

APPENDIX I: INDIVIDUAL CYCLE HISTORIES FROM THE 2-MONTH STUDY (continued)



Legend: Same as APPENDIX I, page 126

APPENDIX I: INDIVIDUAL CYCLE HISTORIES FROM THE 2-MONTH STUDY (continued)



Legend: Same as APPENDIX I, page 126

APPENDIX I: INDIVIDUAL CYCLE HISTORIES FROM THE 2-MONTH STUDY (continued)

DAYS POSTLESION

ANIMAL NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
SHAM - AL																																																															
SE4				X				X				X				X				X				X				X				X				X				X				X				X				X				X				X		
SE14														X											X				X				X				X				X				X				X				X				X		
SE43												X	X				X				X				X				X				X				X				X				X				X				X				X		
SE47														X				X	X				X	X				X				X	X				X	X	X				X	X				X	X				X				X		
SG8												X	X			X	X				X				X				X				X				X				X				X				X				X				X		
SG10											X				X	X				X				X				X				X				X				X				X				X				X				X		
SG16				X				X				X				X				X				X				X	X				X	X				X				X				X				X				X		
SG17				X				X				X				X				X			X	X				X				X				X				X				X				X				X				X		
SG11				X				X			X	X			-	X			X	X				X			X	X			X	X				X				X				X				X				X				X		
SG9				X				X				X				X			X	X				X			X	X			X	X				X				X	X		X	X			X	X				X	X			X	X		
SG13				X				X				X				X					X				X				X				X				X				X				X				X				X				X		
SG21																X			X	X				X				X				X				X			X	X				X				X				X				X		
LESION - AL																																																															
SE19															X				X					X				X				X				X				X				X				X				X				X		
SE28				X							X				X				X			X				X				X				X				X				X				X				X				X		
SE29													X				X				X				X				X				X				X				X				X				X				X		
SE35			X										X			X	X				X			X	X				X				X				X				X				X				X				X		
SE36				X			X	X				X			X	X			X	X			X	X				X			X	X				X				X				X				X				X		
SE38														X				X				X			X				X				X				X				X				X				X				X		
SE49			X					X				X				X				X				X				X				X				X				X				X				X				X		
SE54											X	X					X				X				X				X				X				X				X				X				X				X		
SG6													X			X	X				X				X				X				X				X				X				X				X				X		
SG18											X				X				X			X				X			X	X	X				X				X				X				X				X				X		

Legend: Same as APPENDIX I, page 126

Animal No.	Average Cycle Length 0-30 Days	Average Cycle Length 31-60 Days	Average Cycle Length 0-60 Days
	ESTROUS CYCLE LENGTHS OF CONTROL ANIMALS		
SD4	4.00	4.00	4.00
SD76	4.00	4.00	4.00
SD17	4.00	4.00	4.00
SD48	4.00	4.00	4.00
SD57	4.00	4.00	4.00
SD88	4.00	4.00	4.00
SE15	4.43	4.60	4.58
SE22	4.00	4.00	4.00
SD15	4.14	4.80	4.38
SD79	4.14	5.00	4.46
SE41	4.29	4.83	4.54
SD23	4.14	4.00	4.07
SD38	4.57	4.67	4.62
SD82	4.00	4.00	4.00
SD60	4.14	4.00	4.07
SD74	4.00	4.50	4.21
LG38	4.00	4.17	4.07
SE34	4.00	4.00	4.00
SD3	4.00	4.00	4.00
SD114	4.00	4.00	4.00
	AVERAGE CYCLE LENGTHS OF CONTROL ANIMALS		
SD55	4.00	4.67	4.29
SD36	4.00	4.00	4.00
SD71	4.00	4.17	4.07
SD94	4.00	4.00	4.00
SE7	4.12	4.00	4.09
SE1	4.12	4.14	4.13
SD28	4.14	5.00	4.54
SE53	4.17	5.00	4.78
SE34	4.00	4.50	4.21
	AVERAGE CYCLE LENGTHS OF SHAM ACO-LESIONED ANIMALS		
SD19	4.14	4.00	4.07
SD20	4.14	4.00	4.07
SD49	4.57	5.20	4.83
SD61	4.00	4.00	4.00
SD69	4.14	4.00	4.07
SD116	4.14	4.43	4.29
SE46	4.12	4.00	4.07
SD8	4.29	4.14	4.21
SD99	4.29	4.43	4.36
SD26	4.14	4.00	4.07
SD30	4.00	4.00	4.00
SD42	4.14	4.00	4.07
SD58	4.14	5.17	4.54
SD67	4.00	4.57	4.36
SD90	4.00	4.00	4.00
SE40	4.67	4.17	4.30
SE5	4.29	4.29	4.29
SE25	4.12	4.00	4.07
SD5	4.14	4.00	4.09

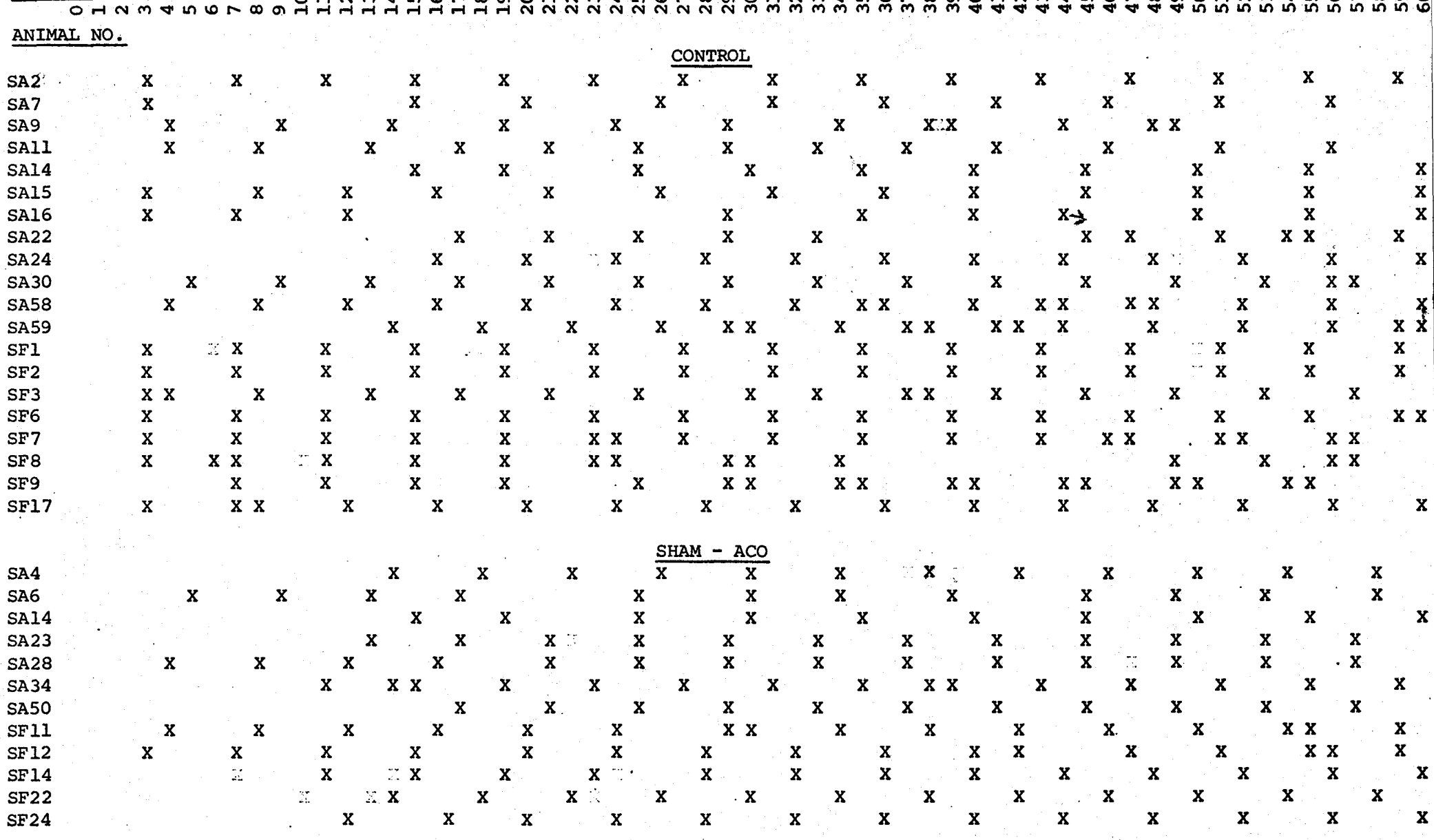
(continued)

Animal No.	Average Cycle Length 0-30 Days	Average Cycle Length 31-60 Days	Average Cycle Length 0-60 Days
	AVERAGE CYCLE LENGTHS OF SHAM ACO-LESIONED ANIMALS (continued)		
SD41	4.14	4.00	4.07
SD65	4.14	4.00	4.07
SD77	4.00	4.00	4.00
SD81	4.00	4.00	4.00
SD109	5.00	4.14	4.25
SE11	4.57	4.33	4.46
SE12	4.00	4.00	4.00
SE27	4.72	4.67	4.69
SD40	4.14	4.00	4.07
SE23	4.57	5.40	4.92
	AVERAGE CYCLE LENGTHS OF ACO-LESIONED ANIMALS		
SD6	4.00	4.00	4.00
SD10	4.14	4.00	4.07
SD22	4.14	4.00	4.07
SD64	4.00	4.00	4.00
SD53	4.29	4.00	4.14
SD105	4.57	5.00	4.75
LG53	4.00	4.33	4.18
SE31	4.00	4.00	4.00
SE26	4.33	4.75	4.67
SE59	4.00	4.00	4.00
SD31	4.00	4.17	4.09
SE21	4.43	5.00	4.58
SE24	4.00	5.00	4.50
SD2	4.14	4.00	4.07
SD95	4.00	4.00	4.00
SD98	4.00	4.00	4.00
SE6	4.25	4.00	4.09
SE51	5.00	4.17	4.14
SD7	4.00	4.67	4.36
SD32	4.29	5.00	4.54
SD59	4.67	4.00	4.20
SD9	4.14	4.00	4.07
SD11	4.00	4.33	4.18
SD50	4.00	4.00	4.00
SD80	4.00	4.33	4.44
SD113	4.14	4.33	4.21
SD120	4.14	4.17	4.14
SE13	4.14	4.00	4.07
SE16	4.00	4.00	4.00
SE2	4.29	4.00	4.14
SE10	4.29	4.00	4.14
	AVERAGE CYCLE LENGTHS OF ST-LESIONED ANIMALS		
SD12	4.00	4.83	4.50
SD21	4.14	4.00	4.07
SD27	4.00	4.29	4.18
SD33	4.29	4.00	4.14
SD35	4.25	4.00	4.09
SD46	4.00	4.67	4.40
SD56	4.29	4.00	4.14
SD66	4.29	4.14	4.21

(continued)

Animal No.	Average Cycle Length 0-30 Days	Average Cycle Length 31-60 Days	Average Cycle Length 0-60 Days
	AVERAGE CYCLE LENGTHS OF ST-LESIONED ANIMALS (continued)		
SD73	4.00	4.00	4.00
SD78	4.00	4.00	4.00
SD83	4.29	4.00	4.14
SD96	4.00	4.00	4.00
SD97	4.00	4.00	4.00
SD100	4.14	4.00	4.07
SD115	5.20	4.80	5.18
	AVERAGE CYCLE LENGTHS OF AL-SHAM LESIONED ANIMALS		
SE4	4.14	4.00	4.07
SE14	5.00	4.00	4.12
SE43	4.00	4.00	4.00
SE47	5.00	5.25	5.14
SG8	4.00	4.00	4.00
SG10	4.25	4.00	4.08
SG16	4.25	4.43	4.36
SG17	4.14	4.00	4.07
SG11	4.14	4.00	4.07
SG9	4.14	4.57	4.36
SG13	4.29	4.00	4.14
SG21	4.00	4.00	4.00
	AVERAGE CYCLE LENGTHS OF AL-LESIONED ANIMALS		
SE19	4.33	4.12	4.18
SE28	4.00	4.12	4.08
SE29	4.00	4.00	4.00
SE35	4.00	4.00	4.00
SE36	4.14	4.00	4.07
SE38	4.00	5.00	4.60
SE49	4.14	3.86	4.14
SE54	4.25	4.00	4.08
SG6	4.00	4.00	4.00
SG18	4.00	4.00	4.00

APPENDIX II: INDIVIDUAL CYCLE HISTORIES FROM THE 4-MONTH STUDY



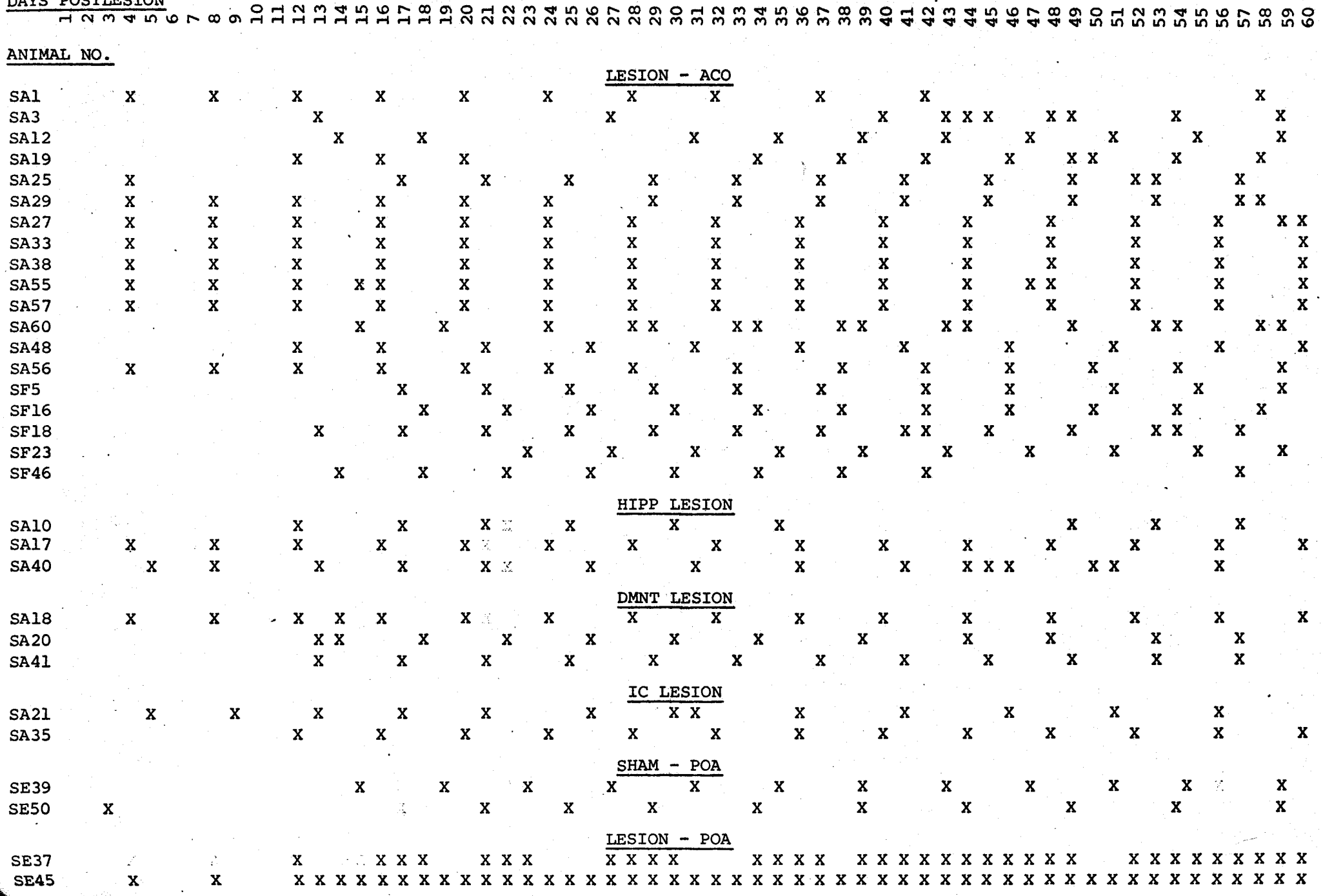
Legend: Same as APPENDIX I

APPENDIX II: INDIVIDUAL CYCLE HISTORIES FROM THE 4-MONTH STUDY (continued)

DAYS POSTLESION

ANIMAL NO.	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120
CONTROL (continued)																																																															
SA2			X				X					X				X					X					X					X					X					X					X					X					X					X	
SA7		X											X	X									X		X											X	X																									
SA9				X	X				X	X						X					X																	X					X																				
SA11	X					X				X	X				X	X				X	X				X	X				X	X					X	X																									X
SA14					X					X				X						X	X					X					X					X																										
SA15					X					X					X					X					X				X	X				X	X					X	X																				
SA16					X					X					X				X	X				X	X				X	X																																	X
SA22		X	X			X				X	X				X				X				X				X				X																														
SA24				X			X				X	X				X	X					X				X					X				X																										
SA30	X			X	X				X			X	X				X				X				X	X				X	X						X																								X
SA58				X			X	X				X				X				X					X				X				X	X																										
SA59				X				X				X	X				X	X				X	X				X	X																																		
SF1			X				X				X					X					X				X					X				X	X	X					X																			
SF2			X				X				X				X				X				X				X				X																														
SF3	X				X	X			X				X	X			X				X	X				X				X				X																										
SF6				X				X				X	X				X	X					X				X				X																														
SF7		X				X									X				X				X				X				X																														
SF8	X				X				X				X				X				X	X				X				X				X																										
SF9										X				X				X					X				X				X	X																													
SF17					X				X				X				X				X	X				X	X				X					X																								
SHAM - ACO (continued)																																																															
SA4		X				X				X				X				X				X				X					X				X					X					X					X	X				X	X			
SA6		X				X				X				X				X				X				X					X				X					X					X					X	X				X	X			X
SA14					X					X				X						X	X					X					X					X																									
SA23	X				X				X				X				X				X					X				X					X					X					X					X					X				
SA28	X			X	X				X				X				X				X					X				X			X	X					X					X					X					X	X			
SA34			X			X				X				X					X	X												X					X					X					X					X	X			
SA50	X			X	X				X	X					X	X				X	X					X	X				X			X	X					X	X																			
SF11			X			X				X					X				X				X					X				X					X					X					X					X	X							X
SF12				X	X				X	X				X	X				X	X				X				X				X	X					X	X				X					X					X	X							X
SF14				X			X				X					X					X	X				X	X				X					X	X					X					X					X	X							
SF22		X			X			X				X			X				X				X				X				X					X					X					X					X	X							X
SF24				X			X				X	X				X				X				X	X				X					X	X					X	X																			

APPENDIX II: INDIVIDUAL CYCLE HISTORIES FROM THE 4-MONTH STUDY (continued)



APPENDIX II: INDIVIDUAL CYCLE HISTORIES FROM THE 4-MONTH STUDY (continued)

DAYS POSTLESION / ANIMAL NO.	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120
LESION - ACO (continued)																																																												
SA1		X				X				X				X				X				X				X				X				X				X				X				X				X				X				X		
SA3				X				X	X			X	X	X				X	X				X	X				X	X				X	X			X	X	X			X	X	X				X	X			X	X	X				X		
SA12			X				X				X				X					X					X				X	X	X	X				X				X	X				X	X			X	X		X			X			X		
SA19		X				X				X				X				X				X				X				X			X	X				X				X			X	X			X	X			X	X			X	X		
SA25	X				X				X				X				X				X				X				X			X	X				X								X				X				X				X			
SA29		X				X				X				X				X				X				X				X			X	X				X				X				X					X					X				
SA27				X				X				X				X			X	X				X			X	X				X				X			X	X			X	X				X				X				X	X			
SA33				X				X				X				X				X				X				X				X				X	X	X				X						X					X							
SA38				X				X			X	X				X				X				X				X				X				X				X				X				X				X				X				X
SA55				X				X				X				X				X				X				X				X				X				X				X					X					X						X
SA57				X				X				X				X				X				X				X				X				X				X				X					X					X				X	X	
SA60				X				X	X				X	X					X					X						X						X				X					X					X					X					
SA48	X	X					X					X					X					X					X					X					X					X					X					X								
SA56				X				X				X				X					X					X					X					X					X					X					X					X				
SF5			X				X				X				X	X				X	X				X				X				X	X														X			X	X				X	X			
SF16		X				X				X				X				X	X				X				X				X				X				X				X				X				X				X				X	
SF18									X	X				X				X				X				X				X				X				X				X				X				X				X				X		
SF23			X				X				X				X				X				X				X				X				X				X				X	X				X		X	X	X				X				X
SF46		X				X				X				X				X				X				X				X				X				X				X				X				X				X				X		
HIPP LESION (continued)																																																												
SA10	X				X				X			X	X					X				X			X	X				X				X			X	X				X						X				X					X			
SA17				X				X				X				X				X					X					X				X					X				X				X				X		X				X	X		
SA40	X			X	X	X					X					X				X	X					X			X	X					X					X				X	X															X
DMNT LESION (continued)																																																											
SA18				X				X				X				X				X				X				X					X	X				X	X				X	X				X				X				X				X
SA20										X													X				X		X				X				X			X	X				X	X				X	X				X	X				
SA41	X				X	X				X	X					X				X				X				X				X				X				X				X				X				X				X				
IC LESION (continued)																																																											
SA21	X					X					X					X					X				X	X				X	X				X	X				X	X				X	X				X	X					X				
SA35				X				X				X				X				X	X				X	X														X				X					X				X				X			
SHAM - POA (continued)																																																											
SE39			X		X				X					X	X		-	-	X	-	-		X	X				X	X	X				X	X				X	X					X				X	X				X	X				X	X
SE50							X			X	X			X	X		-	-	X	-	-			X					X					X					X					X			-	-	X					X					X	
LESION - POA (continued)																																																											
SE37					X	X	X	X			X	X	X	X	X	X	-	-	X	-	-	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	-	-	X	X	X	X	X	X	X	X	X	X	X	

THE FOLLOWING ABBREVIATIONS ARE USED CONSISTENTLY IN APPENDICES III, IV, AND V:

BW.....Body Weight (gm)

AP.....Anterior Pituitary Weight (mg)

Thy.....Thyroid Weight (mg)

Adr.....Adrenal Weight (mg)

Ovr.....Ovarian Weight (mg)

UWW.....Uterine Wet Weight (mg)

UDW.....Uterine Dry Weight (mg)

% DW....Percent Uterine Dry Weight

ULF.....Uterine Lumenal Fluid (mg)

Ova.....Number of Eggs Found in Single Oviduct

Age.....Chronological Age of Animal (Days)

DPO.....Number of Days Between Lesioning or Sham-Lesioning and Autopsy, "Days Post-Operative" (Days)

CLA.....Cycle Length at Autopsy

APPENDIX III: AUTOPSY DATA FROM INDIVIDUAL RATS IN THE 3-CYCLE STUDY

Animal No.	BW	AP	Thy	Adr	Ovr	UWW	UDW	% DW	ULF	Ova	Age	DPO	CLA
CONTROL PROESTRUS													
SF50	315	16.5	23.3	40.1	49.8	483.0	93.8	19.42	123.2	---	117	---	4
SF49	320	14.6	19.3	26.3	51.7	716.6	146.3	20.41	264.1	---	117	---	4
SF47	345	15.7	17.6	35.0	31.1	467.0	88.5	18.95	187.0	---	117	---	4
SB32	323	14.2	13.7	28.0	33.6	520.8	---	---	267.0	---	122	---	4
SB38	278	9.6	16.0	27.0	31.1	465.4	---	---	315.0	---	105	---	4
SB12	285	10.7	---	23.2	32.2	399.2	---	---	116.3	---	102	---	4
SB8	280	12.0	---	21.4	31.0	487.4	---	---	343.2	---	100	---	4
SH17	304	7.9	11.8	26.2	31.4	548.2	102.9	18.77	221.2	---	131	---	4
SH36	344	13.9	14.0	37.4	46.4	529.6	108.6	20.50	123.0	---	128	---	5
LG1	---	13.0	---	---	---	638.8	---	---	Yes	---	83	---	4
CONTROL ESTRUS - AM													
SH61	293	13.2	16.1	36.3	36.8	436.1	87.7	20.1	---	5	130	---	4
SF34	290	12.2	14.4	26.0	41.8	417.0	90.1	21.60	---	13	111	---	4
SH27	297	13.2	17.1	31.4	33.6	407.9	80.4	19.71	---	6	122	---	4
SH21	298	13.4	12.5	33.6	35.0	515.5	104.7	20.71	---	9	124	---	4
SH4	319	13.1	16.2	30.5	34.0	564.0	110.0	19.50	---	6	124	---	5
SI49	274	12.8	15.6	25.1	28.2	460.4	95.2	20.67	---	4	140	---	5
SH1	278	18.7	13.1	27.4	37.0	384.3	82.3	21.41	---	4	132	---	4
SF37	335	16.2	16.8	43.4	37.3	428.4	91.1	21.27	---	7	111	---	4
CONTROL ESTRUS - PM													
SB2	280	9.1	11.9	25.8	34.6	413.0	---	---	---	7	109	---	4
SF43	316	15.4	---	38.8	50.9	463.8	95.5	20.59	---	12	119	---	4
SH26	279	13.0	14.2	30.0	36.0	425.6	---	---	---	5	124	---	4
SH58	312	9.6	13.8	28.1	42.1	426.6	91.3	21.40	---	6	127	---	4
SH7	295	14.9	16.6	33.1	46.8	458.0	93.0	20.30	---	6	132	---	4
SI9	260	13.8	15.6	27.8	38.1	348.6	75.3	21.60	---	7	117	---	5
SI20	256	13.3	15.4	29.2	37.4	448.5	91.1	20.31	---	9	115	---	4
SH19	279	16.9	13.0	25.1	25.6	398.3	84.6	21.24	---	11	127	---	4
SB7	280	8.1	---	31.2	44.8	411.0	---	---	---	6	101	---	4
SB21	261	12.2	14.7	27.2	35.4	481.0	---	---	---	6	102	---	4
CONTROL METESTRUS													
SH5	292	13.4	18.4	34.7	38.8	401.1	86.9	21.66	---	---	125	---	4
SH45	314	14.4	15.6	36.6	38.1	296.6	62.0	20.90	---	---	124	---	4
SH55	338	12.4	26.3	35.5	44.1	302.1	60.9	20.15	---	---	124	---	4
SB4	278	6.4	---	36.6	38.4	340.8	---	---	---	---	100	---	4
SB22	230	8.1	---	22.2	37.6	251.4	---	---	---	---	100	---	4
SE57	325	15.2	17.0	30.0	34.4	416.4	82.8	19.88	---	---	103	---	4
SE62	---	13.7	14.9	35.8	37.4	344.6	82.3	23.88	---	---	116	---	4
SF4	310	12.1	18.4	26.0	48.4	271.2	60.7	22.38	---	---	111	---	4
SF25	335	12.6	---	29.4	35.3	327.2	69.3	21.17	---	---	111	---	4
SF35	314	12.3	16.1	24.2	32.4	310.2	71.6	23.08	---	---	111	---	4
SF40	307	15.6	22.1	33.0	37.3	319.6	70.1	21.93	---	---	111	---	4
SF44	300	13.8	13.4	32.8	40.0	326.0	72.9	22.36	---	---	111	---	4
SF38	334	17.8	---	33.8	48.1	450.2	96.1	21.34	---	---	110	---	4
SF41	318	14.8	10.5	30.5	38.0	285.6	61.2	21.42	---	---	110	---	4
SH53	324	14.2	15.0	31.2	38.3	338.4	71.1	21.01	---	---	124	---	4

Abbreviations Same as on Page 138

APPENDIX III: AUTOPSY DATA FROM INDIVIDUAL RATS IN THE 3-CYCLE STUDY (continued)

Animal No.	BW	AP	Thy	Adr	Ovr	UWW	UDW	% DW	ULF	Ova	Age	DPO	CLA
					SHAM - ACO PROESTRUS								
SB9	310	14.9	13.8	37.5	38.2	776.4	---	---	360.8	---	122	11	4
SB10	274	10.4	16.0	24.8	31.8	557.5	---	---	236.1	---	109	11	4
SB11	280	8.1	---	24.4	48.3	580.0	---	---	318.4	---	101	11	5
SB19	298	12.2	18.8	33.4	40.2	446.0	---	---	226.8	---	113	24	4
SH6	265	10.8	20.9	29.5	41.7	561.0	106.0	18.89	322.7	---	110	15	4
SH57	303	11.2	10.7	31.5	39.2	365.0	75.6	20.71	175.5	---	127	22	4
SI13	228	9.2	11.3	25.9	26.0	375.5	68.3	18.18	124.8	---	108	12	4
					SHAM - ACO ESTRUS - AM								
SH34	320	11.8	16.7	26.2	36.8	475.3	95.3	20.03	---	7	142	37	5
SI40	259	11.3	15.4	32.4	41.8	403.3	76.6	18.99	58.2	8	121	24	4
SI43	245	9.1	14.6	39.3	38.3	465.8	83.9	18.01	---	7	130	12	4
SI45	252	7.9	17.0	29.5	34.1	383.9	78.4	20.42	---	6	121	24	4
SI60	244	13.6	22.7	29.9	30.2	491.6	93.0	18.91	---	9	126	14	5
SI17	279	14.1	15.0	30.0	44.5	393.4	---	---	---	4	141	24	4
SI56	268	11.4	32.2	37.4	37.8	492.6	99.6	20.22	---	2	126	14	5
					SHAM - ACO ESTRUS - PM								
SB37	312	11.7	16.1	34.0	37.6	391.2	---	---	---	8	128	30	5
SB34	310	13.6	12.0	35.5	32.0	495.2	---	---	---	7	139	27	4
SH41	---	16.0	17.8	44.0	41.1	473.2	95.8	20.24	---	7	110	12	4
SH49	310	18.5	22.8	38.0	51.3	458.5	87.3	19.04	---	6	140	36	4
SI36	265	10.6	17.5	31.9	35.5	370.8	75.1	20.25	---	7	130	12	4
SH50	273	14.6	20.2	33.3	42.0	409.2	81.8	19.99	---	8	110	12	4
SH35	318	10.2	19.4	33.4	47.4	423.4	83.4	19.69	---	Yes	118	12	4
SB16	316	14.8	16.0	32.4	48.2	418.0	---	---	---	8	114	23	4
					SHAM - ACO METESTRUS								
SB28	252	6.1	13.4	28.4	35.0	308.8	---	---	---	---	104	13	4
SB15	312	12.7	14.2	33.8	41.0	353.4	---	---	---	---	116	26	4
SB40	317	10.8	21.8	37.0	43.4	335.2	---	---	---	---	123	25	4
SH13	268	14.7	---	37.4	40.8	305.0	65.1	21.34	---	---	109	13	4
SH48	256	12.3	22.1	30.0	36.1	264.2	55.4	20.96	---	---	109	13	4
SH28	295	9.8	20.1	36.5	33.0	466.7	95.1	20.37	---	---	110	36	5
SH22	---	15.0	21.0	30.1	35.9	438.7	89.4	20.37	---	---	118	13	4

Abbreviations Same as on Page 138
(continued)

APPENDIX III: AUTOPSY DATA FROM INDIVIDUAL RATS IN THE 3-CYCLE STUDY (continued)

Animal No.	BW	AP	Thy	Adr	Ovr	UWW	UDW	% DW	ULF	Ova	Age	DPO	CLA
					LESION - ACO PROESTRUS								
SB20	290	15.3	15.4	30.1	35.2	486.4	---	---	273.8	---	113	24	4
SB27	310	16.6	15.4	29.6	37.2	558.6	---	---	584.0	---	114	24	4
SB39	330	15.2	19.4	35.2	48.6	506.2	---	---	Yes	---	119	23	4
SB42	335	13.4	16.0	42.0	35.3	571.6	---	---	331.0	---	129	11	4
SI44	262	9.4	12.1	36.2	37.2	393.4	79.9	20.31	159.3	---	106	11	4
SI54	230	12.4	19.8	25.8	26.6	562.7	108.0	19.19	110.0	---	108	11	4
SI29	250	9.4	17.2	31.0	21.6	518.9	94.6	18.23	120.1	---	110	13	4
SI18	283	9.4	14.2	33.9	46.6	441.6	83.7	18.95	155.2	---	120	23	4
SI32	275	10.5	18.5	30.8	36.6	484.1	93.2	19.25	155.6	---	120	25	4
					LESION - ACO ESTRUS - AM								
SH12	318	12.2	19.6	42.1	35.6	491.0	101.7	20.71	---	5	122	27	4
SH18	361	16.4	15.1	32.9	39.5	503.3	107.3	21.31	---	7	130	25	4
SI34	268	13.6	18.5	35.4	49.0	452.0	87.1	19.26	---	6	120	24	4
SI6	266	12.2	13.5	26.5	39.1	322.4	64.7	20.06	---	6	139	25	4
SI2	269	10.2	19.5	26.4	34.2	444.1	89.3	20.10	---	6	121	24	4
SI1	268	9.0	12.3	29.9	31.9	394.9	80.0	20.25	---	5	118	23	4
SI8	282	11.4	17.1	32.1	30.3	412.2	---	---	---	4	151	37	5
					LESION - ACO ESTRUS - PM								
SB24	313	19.6	16.0	29.2	46.4	440.6	---	---	---	6	128	39	4
SB1	319	12.0	11.4	28.1	31.2	330.8	---	---	---	6	140	42	4
SH54	295	13.9	16.5	42.1	57.4	420.0	90.6	21.57	---	9	109	12	4
SH44	315	9.8	16.6	34.0	47.4	447.5	92.6	20.69	---	7	122	25	4
SI28	260	9.4	16.8	30.2	42.1	307.5	64.3	20.87	---	3	122	25	4
SI31	253	10.4	13.4	34.4	30.8	384.2	77.0	20.04	---	6	121	24	4
SI33	255	12.4	17.2	26.2	40.0	565.2	112.4	19.88	---	6	121	25	4
SI3	284	11.4	13.5	28.0	37.6	399.2	77.7	19.46	---	10	141	27	4
SI41	275	13.2	21.2	28.3	45.2	521.3	103.2	19.79	---	11	128	24	4
SB25	308	10.1	11.8	36.2	38.4	432.4	---	---	---	7	105	13	4
					LESION - ACO METESTRUS								
SB35	283	14.9	17.4	37.8	40.6	303.4	---	---	---	---	109	13	4
SB26	286	13.2	15.2	31.2	47.6	282.4	---	---	---	---	115	26	4
SB18	319	13.6	15.4	29.6	33.2	312.0	---	---	---	---	139	28	4
SH37	310	13.4	15.9	36.2	42.1	327.7	67.2	20.51	---	---	122	25	4
SH42	346	12.0	17.1	34.3	37.8	358.2	78.6	21.94	---	---	127	30	4
SI24	238	7.2	11.7	21.5	23.2	310.1	66.1	21.32	---	---	110	13	4
SI47	238	6.7	12.2	33.2	42.1	222.0	49.5	22.30	---	---	108	13	4
SI57	253	9.2	16.2	28.1	22.3	309.4	66.9	21.62	---	---	111	14	5
SI12	268	11.6	13.8	36.2	34.1	374.8	77.8	20.76	---	---	121	24	4
SI37	290	12.9	20.5	33.4	45.2	347.4	69.9	20.12	---	---	125	28	4

Abbreviations Same as on Page 138

APPENDIX IV: AUTOPSY DATA FROM INDIVIDUAL RATS IN THE 2-MONTH STUDY

Animal No.	BW	AP	Thy	Adr	Ovr	UWW	UDW	% DW	ULF	Ova	Age	DPO	CLA
CONTROL PROESTRUS													
SD4	305	17.2	17.3	24.6	44.3	712.1	131.1	18.41	400.4	---	139	---	4
SD76	304	16.8	14.0	24.6	26.1	562.3	106.6	18.95	205.0	---	143	---	4
SD17	288	18.5	22.3	26.2	44.7	589.2	103.4	17.54	294.5	---	140	---	4
SD48	324	14.5	20.4	31.4	33.8	709.8	125.2	17.63	374.2	---	140	---	4
SD57	317	18.6	17.3	28.1	32.5	666.4	124.2	18.64	209.0	---	146	---	4
SD88	295	14.3	11.3	25.0	39.2	487.4	92.1	18.89	352.3	---	165	---	4
SE15	284	15.2	13.2	33.5	33.0	527.4	94.2	17.86	197.9	---	151	---	5
SE22	315	16.5	16.0	26.4	35.8	828.6	141.3	17.05	165.4	---	150	---	4
CONTROL ESTRUS - AM													
SD23	330	18.9	20.3	37.8	52.4	584.4	99.3	16.99	---	7	138	---	4
SD38	288	15.2	14.7	29.4	36.8	478.5	97.4	20.35	---	4	180	---	4
SD82	320	11.3	14.2	31.9	41.0	399.2	77.5	19.41	---	8	158	---	4
SD60	262	11.3	14.0	19.5	27.2	380.1	68.1	17.91	---	9	138	---	4
SD74	312	17.5	19.0	25.5	50.9	552.1	108.1	19.57	---	4	150	---	4
LG38	348	18.0	22.0	30.1	38.2	554.2	108.9	19.64	---	5	155	---	4
SE34	313	22.0	13.7	33.7	34.4	619.6	119.1	19.22	---	9	158	---	4
SD3	306	16.4	14.2	28.4	33.4	433.2	85.0	19.62	---	4	141	---	4
SD114	324	14.4	16.2	25.2	40.6	450.2	387.9	19.52	---	7	153	---	4
CONTROL ESTRUS - PM													
SD55	282	18.7	22.4	27.8	31.6	507.4	94.2	18.56	---	5	141	---	5
SD36	315	19.2	22.3	35.7	39.0	480.1	89.0	18.53	---	7	141	---	4
SD71	306	21.2	17.5	29.7	40.6	507.6	96.5	19.01	---	8	138	---	4
SD94	358	17.4	24.7	43.4	40.1	525.8	96.4	18.33	---	8	166	---	4
SE7	304	15.0	16.0	26.5	38.1	349.6	73.6	21.05	---	5	160	---	5
SE1	348	18.6	14.1	34.6	33.5	469.8	91.5	19.47	---	7	155	---	5
SD28	324	18.8	15.8	34.3	30.6	686.7	125.4	18.26	---	5	151	---	4
SE53	325	12.4	13.5	20.3	30.1	357.3	73.8	20.65	---	6	158	---	5
SE34	316	16.6	12.4	25.2	43.2	648.0	119.0	18.36	---	9	158	---	4

Abbreviations same as on page 138
(continued)

APPENDIX IV: AUTOPSY DATA FROM INDIVIDUAL RATS IN THE 2-MONTH STUDY (continued)

Animal No.	BW	AP	Thy	Adr	Ovr	UWW	UDW	% DW	ULF	Ova	Age	DOP	CLA
					SHAM - ACO PROESTRUS								
SD19	309	13.1	17.8	28.2	28.3	658.3	117.6	17.86	319.4	---	141	59	4
SD20	305	14.8	20.2	27.8	27.4	712.3	125.4	17.60	193.9	---	142	59	4
SD49	291	16.0	17.9	29.8	21.7	478.0	91.1	19.05	455.8	---	143	61	5
SD61	305	14.0	20.4	27.3	32.3	863.7	155.4	17.99	292.1	---	149	61	4
SD69	305	18.3	20.9	27.6	25.3	752.9	138.2	18.35	522.1	---	146	59	4
SD116	346	17.3	21.2	30.5	35.0	1052.9	202.1	19.19	1692.2	---	165	63	5
SE46	324	18.8	20.4	34.7	36.8	829.5	145.1	17.49	765.8	---	153	59	4
					SHAM - ACO ESTRUS - AM								
SD26	301	18.1	17.3	28.4	37.2	628.2	106.7	16.98	---	8	138	57	4
SD30	300	13.3	21.1	30.3	35.8	601.5	113.4	18.85	---	9	141	59	4
SD42	348	17.3	13.2	31.9	42.0	539.6	96.1	17.80	---	5	141	60	4
SD58	314	17.4	16.3	28.2	42.0	589.6	109.1	18.50	---	6	140	59	5
SD67	330	19.0	15.9	32.0	38.1	419.4	85.9	20.48	---	4	152	65	5
SD90	334	17.0	15.9	31.3	36.5	707.0	134.1	18.96	---	7	148	61	4
SE40	320	16.6	19.6	29.4	38.4	519.3	93.9	18.08	---	7	155	64	5
SE5	288	13.4	13.7	23.8	32.0	405.2	86.1	21.24	---	1	165	73	4
SE25	320	15.6	21.1	32.4	42.9	484.7	88.1	18.17	---	6	152	60	4
					SHAM - ACO ESTRUS - PM								
SD5	312	11.7	18.5	26.3	52.5	397.3	77.7	19.55	---	5	152	70	4
SD41	289	14.4	19.5	29.4	40.8	614.4	116.0	18.88	---	9	142	60	4
SD65	310	18.2	19.5	28.3	52.1	478.2	92.8	19.40	---	4	142	60	4
SD77	326	16.2	17.5	28.5	37.4	453.8	92.1	20.29	---	11	155	60	4
SD81	296	16.2	14.4	27.6	34.7	438.0	84.0	19.17	---	6	145	59	4
SD109	279	12.6	15.2	31.6	30.1	410.0	84.4	20.58	---	7	155	60	4
SE11	315	17.3	15.4	28.9	31.2	566.1	108.5	19.16	---	5	149	57	5
SE12	304	12.6	12.4	29.7	32.3	417.2	81.0	19.41	---	5	153	60	4
SE27	320	16.4	14.7	35.5	46.0	482.6	91.1	18.87	---	7	153	60	4
SD40	281	11.7	21.3	27.6	---	708.2	136.7	19.30	---	6	143	60	4
SE23	303	17.4	13.7	27.0	26.4	620.1	122.7	19.78	---	11	158	63	5

Abbreviations same as on page 138

(continued)

APPENDIX IV: AUTOPSY DATA FROM INDIVIDUAL RATS IN THE 2-MONTH STUDY (continued)

Animal No.	BW	AP	Thy	Adr	Ovr	UWW	UDW	% DW	ULF	Ova	Age	DOP	CLA
					LESION - ACO PROESTRUS								
SD6	305	10.9	20.8	27.2	25.9	555.0	101.5	18.28	---	---	138	55	4
SD10	296	11.7	15.5	25.2	36.8	592.2	101.7	17.17	202.4	---	140	59	4
SD22	284	11.2	15.6	27.6	34.0	520.4	92.0	17,67	214.3	---	142	59	4
SD64	316	15.6	16.5	37.0	36.7	735.8	138.2	18.78	537.6	---	148	60	4
SD53	346	17.8	15.8	27.6	36.4	723.5	138.6	19.15	346.2	---	153	65	5
SD105	321	20.0	21.4	37.4	38.9	795.5	150.9	18.96	225.0	---	167	65	5
LG53	322	16.5	18.7	35.2	40.9	485.7	94.9	19.53	212.4	---	155	65	5
SE31	309	15.8	20.9	27.6	37.6	609.0	103.2	16.94	249.0	---	151	58	4
SE26	362	19.1	23.6	31.0	41.8	669.3	117.9	17.61	80.2	---	151	59	5
SE59	300	14.9	22.5	27.4	31.6	533.7	93.2	17.46	76.0	---	153	59	4
					LESION - ACO ESTRUS - AM								
SD2	320	13.9	17.3	22.1	28.2	351.6	68.5	19.48	---	6	138	57	4
SD95	342	15.1	15.1	30.0	45.3	448.0	92.0	20.53	---	4	157	70	4
SD98	328	15.9	18.8	21.0	40.6	465.2	95.6	20.55	---	5	144	59	4
SE6	324	19.1	17.5	29.5	33.4	610.9	114.2	18.69	---	8	149	57	4
SE51	369	20.4	20.8	30.0	43.3	425.3	82.5	19.39	---	6	149	57	5
SD7	330	11.4	20.6	26.6	28.7	676.4	135.6	20.04	---	6	143	60	5
SD32	304	16.6	19.0	24.0	31.3	450.0	86.1	19.13	---	1	141	58	5
SD59	329	18.3	16.7	27.7	37.5	613.5	121.5	19.80	---	3	159	60	4
					LESION - ACO ESTRUS - PM								
SD9	307	20.3	22.0	32.8	33.0	567.8	103.3	18.19	---	5	142	60	4
SD11	348	19.6	19.0	31.5	33.1	470.4	87.3	18.55	---	7	141	58	4
SD50	310	11.8	16.6	29.6	30.1	552.5	105.1	19.02	---	7	143	60	4
SD80	296	18.0	19.6	32.8	37.2	401.5	75.0	18.67	---	12	145	59	4
SD113	367	21.0	18.1	35.4	48.4	551.0	100.8	18.29	---	7	161	62	4
SD120	320	21.1	16.8	37.0	45.0	645.3	127.0	19.68	---	11	164	61	4
SE13	330	15.5	13.4	35.6	52.7	562.7	102.8	18.26	---	10	153	60	4
SE16	312	18.2	15.8	38.2	46.3	493.4	86.7	17.57	---	6	149	58	4
SE2	275	19.9	20.0	31.3	28.3	530.2	104.9	19.78	---	6	160	65	4
SE10	362	13.1	16.0	24.5	43.8	471.6	91.6	19.42	---	8	160	65	4

Abbreviations same as on page 138
(continued)

APPENDIX IV: AUTOPSY DATA FROM INDIVIDUAL RATS IN THE 2-MONTH STUDY (continued)

Animal No.	BW	AP	Thy	Adr	Ovr	UWW	UDW	% DW	ULF	Ova	Age	DOP	CLA
					SHAM - "ST" ESTRUS - AM								
SD13	300	17.7	---	30.1	40.1	488.2	101.7	20.83	---	7	152	62	5
SD14	278	13.2	14.5	28.0	35.8	409.3	78.5	19.17	---	4	153	64	4
SD44	304	18.5	16.2	33.0	36.2	637.4	---	---	---	9	153	64	4
SD84	274	11.7	12.2	24.8	39.1	492.3	99.5	20.21	---	6	155	60	4
SD104	318	13.7	14.3	37.3	29.0	395.6	80.3	20.29	---	8	155	60	4
SD108	323	14.2	15.0	40.1	37.2	450.2	90.9	20.19	---	7	156	61	4
SD117	318	16.1	20.3	31.3	26.6	731.1	138.3	18.91	---	7	160	61	4
SE17	382	15.9	7.3	29.8	41.2	653.4	123.0	18.82	---	5	155	61	4
SE44	339	23.0	29.2	41.2	46.3	572.1	107.0	18.70	---	3	155	61	5
					LESION - "ST" PROESTRUS								
SD21	305	13.4	18.2	31.7	38.4	486.5	88.2	18.12	206.8	---	141	59	4
SD73	332	14.6	20.3	31.5	33.1	684.2	124.4	18.18	399.0	---	149	61	4
SD78	306	17.7	14.4	24.2	37.4	587.0	108.1	18.41	318.9	---	146	59	4
SD97	325	19.5	18.8	26.3	49.2	480.0	89.9	18.72	170.6	---	146	59	4
SD56	302	14.9	20.2	28.6	36.0	716.2	132.5	18.50	641.1	---	148	60	4
					LESION - "ST" ESTRUS - AM								
SD33	329	15.2	16.2	24.8	40.4	472.4	97.4	20.61	---	4	144	61	4
SD35	322	17.3	17.8	27.1	37.6	358.9	75.8	21.12	---	7	142	59	4
SD96	296	14.9	18.1	30.0	40.9	607.5	116.4	19.16	---	6	148	61	4
SD100	332	17.5	15.4	24.2	29.2	519.0	107.5	20.71	---	8	146	60	4
SD115	320	16.8	16.6	28.4	35.5	514.0	102.0	19.84	---	8	160	61	5
					LESION - "ST" ESTRUS - PM								
SD83	348	11.9	17.3	27.8	43.7	428.9	89.0	20.75	---	6	151	65	4
SD66	325	18.2	15.0	24.6	26.7	414.0	77.8	18.79	---	9	145	62	4
SD46	328	19.2	13.8	38.7	33.0	400.0	82.5	20.62	---	6	144	62	5
SD27	335	19.1	15.3	39.1	34.1	627.4	123.3	19.65	---	6	145	62	4
SD12	275	14.1	11.3	24.0	25.6	388.1	77.8	20.04	---	4	159	63	5

Abbreviations same as on page 138
(continued)

APPENDIX IV: AUTOPSY DATA FROM INDIVIDUAL RATS IN THE 2-MONTH STUDY (continued)

Animal No.	BW	AP	Thy	Adr	Ovr	UWW	UDW	% DW	ULF	Ova	Age	DOP	CLA
						SHAM - AL PROESTRUS							
SE4	322	16.8	17.8	27.8	31.2	666.3	116.9	17.54	436.0	---	158	59	4
SG8	294	11.8	18.0	24.6	31.7	683.0	125.0	18.30	341.2	---	162	64	4
SG10	285	12.5	18.4	30.4	39.2	718.3	129.2	17.98	218.6	---	166	63	4
SG16	321	12.6	15.8	30.1	25.2	506.1	92.7	18.31	907.9	---	160	60	5
SG17	318	13.4	19.7	35.7	59.0	523.0	103.9	19.86	384.2	---	166	63	4
						SHAM - AL ESTRUS - AM							
SE14	297	14.5	16.0	21.4	35.0	420.9	81.0	19.24	---	7	158	59	4
SE43	300	16.7	13.8	28.2	36.4	757.2	141.7	18.71	---	7	164	61	4
SG9	338	13.4	16.0	30.2	26.2	482.1	92.7	19.21	---	8	163	60	5
SG11	324	13.5	24.5	27.5	37.3	573.2	109.8	19.15	---	9	165	64	4
SG21	319	14.5	20.0	32.1	37.2	485.6	87.4	17.99	---	9	165	64	4
SG13	328	15.3	20.0	41.8	33.2	427.4	81.0	18.95	---	6	174	62	5
						LESION - AL PROESTRUS							
SE28	300	12.7	17.4	26.0	33.2	566.0	97.7	17.22	288.4	---	158	63	4
SE36	298	19.0	13.7	19.8	25.1	648.1	112.8	17.40	517.8	---	159	59	4
SE49	299	15.5	15.8	30.3	38.2	731.3	138.7	18.96	448.7	---	162	59	4
SG6	292	13.2	16.5	24.5	37.2	527.4	92.2	17.46	123.6	---	160	60	4
SG18	270	12.8	14.3	28.2	34.7	500.6	85.6	17.09	285.1	---	160	58	4
						LESION - AL ESTRUS - AM							
SE35	331	14.4	14.5	25.8	41.5	519.8	105.5	20.29	---	5	160	60	4
SE38	387	21.0	18.4	33.7	39.2	501.4	99.0	19.74	---	10	164	65	5
SE19	327	12.7	13.4	28.0	32.3	371.4	77.0	20.73	---	5	163	61	4
SE54	322	15.7	12.4	25.7	36.0	546.6	107.6	19.69	---	8	164	61	4

Abbreviations same as on page 138

APPENDIX V: AUTOPSY DATA FROM INDIVIDUAL RATS IN THE 4-MONTH STUDY

Animal No.	BW	AP	Thy	Adr	Ovr	UWW	UDW	% DW	ULF	Ova	Age	DOP	CLA
						CONTROL PROESTRUS							
SA11	352	15.9	28.1	33.3	47.8	710.6	---	---	224.8	---	209	---	5
SA24	278	13.2	24.4	24.0	39.3	608.6	---	---	612.0	---	208	---	5
SA30	407	12.0	12.3	26.7	39.2	563.6	---	---	---	---	212	---	4
SA58	397	---	12.0	---	42.4	495.6	106.0	21.39	---	---	205	---	5
SA59	415	9.0	17.3	38.2	37.5	676.6	129.0	19.07	538.1	---	234	---	5
SF2	365	14.6	29.7	31.9	45.5	598.8	105.8	17.67	483.2	---	240	---	4
SF3	388	20.0	18.5	29.6	42.3	559.3	115.8	20.70	359.5	---	250	---	4
SF19	354	20.6	25.1	35.7	35.4	906.8	---	---	965.9	---	242	---	5
						CONTROL ESTRUS - PM							
SA7	417	14.4	19.4	29.6	48.4	390.6	---	---	---	9	239	---	5
SA15	355	19.4	19.2	37.2	37.7	468.9	---	---	---	5	230	---	4
SA22	402	17.4	18.7	26.0	53.8	373.4	72.3	19.36	---	5	231	---	4
SF7	380	26.6	15.4	38.6	42.2	672.0	147.1	21.89	---	9	244	---	4
SF8	314	15.0	19.3	22.6	34.8	559.7	118.8	21.23	---	5	247	---	4
						SHAM - ACO PROESTRUS							
SA14	358	13.8	26.6	27.6	38.4	483.4	---	---	187.4	---	248	129	5
SA23	390	13.4	18.5	31.2	35.5	701.8	131.9	18.79	100.6	---	221	119	5
SA28	323	11.6	17.3	34.1	49.8	573.5	---	---	Yes	---	210	120	4
						SHAM - ACO ESTRUS - PM							
SA50	400	17.2	22.8	39.8	44.3	558.4	104.6	18.73	---	15	202	116	5
SF11	350	15.3	28.4	27.7	38.8	602.9	116.6	19.34	---	Yes	240	121	5
SF12	352	21.4	14.8	32.4	51.8	755.2	169.4	22.43	---	9	243	140	5
SF14	364	17.7	22.2	29.4	40.2	467.3	105.3	22.53	---	7	242	122	5
SF22	425	20.0	25.6	31.4	45.8	509.0	111.5	21.91	---	7	243	121	5

Abbreviations same as on page 138
(continued)

APPENDIX V: AUTOPSY DATA FROM INDIVIDUAL RATS IN THE 4-MONTH STUDY (continued)

Animal No.	BW	AP	Thy	Adr	Ovr	UWW	UDW	% DW	ULF	Ova	Age	DOP	CLA
					LESION - ACO PROESTRUS								
SA19	352	11.6	15.1	23.8	31.4	491.6	---	---	151.0	---	223	125	4
SA29	316	12.0	19.2	28.6	37.0	539.6	---	---	Yes	---	210	120	5
SA33	336	14.4	17.8	42.6	42.0	648.6	---	---	1054.4	---	214	117	5
SA38	336	13.4	19.4	28.7	49.8	726.0	129.0	17.77	374.1	---	219	119	4
SA57	385	7.2	14.4	18.4	34.2	759.0	129.2	17.02	Yes	---	206	123	5
					LESION - ACO ESTRUS - AM								
SA25	336	16.7	17.2	29.7	40.2	492.8	92.1	18.96	---	10	216	121	4
SA27	356	13.6	21.4	38.9	44.0	457.4	92.2	20.16	---	7	219	122	5
SA48	373	13.4	25.0	28.4	42.7	523.6	104.2	19.90	---	4	202	112	5
SA56	369	15.8	24.1	40.8	39.2	600.1	113.6	18.93	---	9	203	116	5
					LESION - ACO ESTRUS - PM								
SA1	363	20.0	10.4	25.2	35.6	487.2	---	---	---	7	211	122	4
SA3	440	25.0	14.0	36.2	62.6	636.6	---	---	---	9	209	118	4
SA51	342	16.7	21.9	35.8	31.6	489.0	98.1	20.06	---	4	203	120	4
SA55	360	9.9	11.8	27.5	44.8	551.0	103.2	18.73	---	6	232	132	4
SA60	397	19.1	17.8	25.2	41.2	418.0	84.0	20.10	---	9	202	115	5
SF5	330	16.3	19.1	31.5	40.0	437.8	91.5	20.90	---	6	243	121	4
SF16	344	14.6	32.2	29.6	34.6	524.1	91.5	17.46	---	4	240	123	4
SF18	415	16.0	18.6	32.1	46.2	721.8	156.6	21.70	---	9	241	122	4
SF46	292	17.1	16.2	25.6	24.5	504.5	103.8	20.57	---	7	243	126	4
SF23	422	23.3	23.2	44.6	48.1	730.0	140.8	19.29	---	8	247	125	4

Abbreviations same as on page 138

	Animal No.	Assay No.	Plasma LH µg/2 ml	Plasma LH ng/ml
		CONTROL		
PROESTRUS	LG42	C8	.112	56.0
	LG24	C8	.140	70.0
	LG74	C21	.088	44.0
	LG75	C21	.039	18.5
	LG77	C21	.024	12.0
	LG72	C23	.037	16.5
	LG89	C23	.035	17.5
	LG90	C23	.030	15.0
	SH36	D7	.053	26.5
	SH17	D13	.030	15.0
	SB8 & 12	B34	.099	49.5
	SB12	B34	.000†	0.0
	SB38	B34	.051	25.5
	SB32	B34	.025	12.5
	SF49	C49	.113	56.5
	SF50	C49	.160	80.0
ESTRUS - AM	LG80	C23	.000	0.0
	SH27	D7	.000	0.0
	SH4	D7	.029	19.5
	SH21	D7	.000	0.0
	SH21 & 4	D7	.034	17.0
	SI49	D18	.037	18.5
	SH61	D13	.000	0.0
	SH1	D13	.029	14.5
	SF34	D13	.056	28.0
ESTRUS - PM	SH26	D7	.038	19.0
	SH19	D7	.000	0.0
	SH58	D7	.055	27.5
	SH7	D13	.040	20.0
	SI20	D13	.000	0.0
	SI9	D13	.000	0.0
	SF43	D49	.000	0.0
	SB2	B34	.000	0.0
	SB7	B37	.038	19.0
METESTRUS	SH45	D7	.029	14.5
	SH53	D7	.071	35.5
	SH55	D7	.000	0.0
	SH5	D7	.064	32.0
	SE57	C21	.180	90.0
	SE62	C21	.190	95.0
	SF38	C49	.035	17.5
	SF41	C49	.186	93.0
	SF40	C49	.038	19.0
	SF44	C49	.000	0.0
	SF4	C49	.094	47.0
	SF25	C49	.177	88.5
	SF35	C49	.000	0.0

†When a plasma sample causes no depletion of ovarian ascorbic acid its LH concentration is assigned a value of .000 µg/2 ml.

‡Omitted by Outlier Critera (26)

(continued)

APPENDIX VI: INDIVIDUAL PLASMA LH VALUES: 3-CYCLE STUDY (continued)

	Animal No.	Assay No.	Plasma LH µg/2 ml	Plasma LH ng/ml
		ACO - SHAM		
PROESTRUS	SH6	D7	.100	50.0
	SH57	D7	.000	0.0
	SI13	D13	.037	18.5
	SB11	B34	.075	37.5
	SB9 & 10	B34	.000	0.0
	SB10	B34	.080	40.0
	SB19	B34	.045	22.5
ESTRUS - AM	SI43	D18	.460	230.0‡
	SI17	D18	.000	0.0
	SH34	D13	.000	0.0
	SI40	D13	.016	8.0
	SI45	D13	.016	8.0
	SI56	D13	.080	40.0
ESTRUS - PM	SH41	D7	.042	21.0
	SH50	D7	.100	50.0
	SH35	D7	.000	0.0
	SH49	D13	.049	24.5
	SB16	B34	.072	36.0
	SB37	B35	.056	28.0
	SB34	B36	6.40 ‡	--
METESTRUS	SH13 & 48	D7	.840	420.0‡
	SH28	D7	.044	22.0
	SH22	D7	.076	38.0
	SB15	B34	.112	56.0
	SB28	B34	.029	14.5
	SB40	B34	.280	140.0
		ACO - LESION		
PROESTRUS	SI44	D7	.025	12.5
	SI54	D13	.480	240.0‡
	SI29	D13	.032	16.0
	SI18	D13	.000	0.0
	SI32	D13	.000	0.0
	SB20	D34	.025	12.5
	SB27	B34	.115	57.5
	SB39	B34	.113	56.5
	SB42	B36	.078	39.0
ESTRUS - AM	SH12	D7	.000	0.0
	SI6	D18	.037	18.5
	SI8	D18	.000	0.0
	SH18	D13	.000	0.0
	SH18	D13	.015	7.5
	SI1	D13	.017	8.5
	SI34	D13	.020	10.0
	SI2	D13	.000	0.0
ESTRUS - PM	SH54	D7	.000	0.0
	SI41	D18	.000	0.0
	SI3	D18	.180	90.0
	SI31	D13	.000	0.0
	SI33	D13	.165	82.5
	SI28	D13	.018	9.0
	SB24	B34	.121	60.5
	SB1	B41	.000	0.0
	SH54	D7	.000	0.0
	SB25	B34	.108	54.0
	SB44	B37	.000	0.0
METESTRUS	SH37	D7	.000	0.0
	SH42	D7	.102	51.0
	SI47	D13	.038	19.0
	SI24	D13	.018	9.0
	SI37	D13	.016	8.0
	SI57	D13	.000	0.0
	SB26	B34	.000	0.0
	SB35	B34	.290	145.0

Legend Same as APPENDIX VI, Page 149 (continued)

	Animal No.	Assay No.	Plasma LH g/2 ml	Plasma LH ng/ml	
		CONTROL			
PROESTRUS:					
2:30-3:30 PM	SD4	C22	.220	110.0	
	SD17	C22	.000	0.0	
	SD48	C22	.000	0.0	
	SD76	C24	.000	0.0	
	SD57	C24	.210	105.0	
	SD88	C27	.053	26.5	
	SE22	C28	.000	0.0	
	SE15	C28	.225	112.5	
PROESTRUS:					AGE
5:00-6:00 PM	SE3	C28	.280	140.0	149
	SE52	C28	.370	185.0	149
	SE42	C28	.480	240.0	151
	SE48	C28	.295	147.5	152
	SE61	C28	.373	186.5	152
	SE55	C28	.500	250.0	152
	SE55,52,61	C28	.520	260.0	---
ESTRUS - AM	SD23	C22	.098	49.0	
	SD3	C22	.087	43.5	
	SD74	C25	.000	0.0	
	SD114	C25	.000	0.0	
	SD74 & 114	C25	.039	19.5	
	SD82	C27	.000	0.0	
ESTRUS - PM	SD71	C22	.110	55.0	
	SD34	C22	.000	0.0	
	SD55	C22	.000	0.0	
	SD34 & 55	C22	.000	0.0	
	SD36	C23	.123	61.5	
	SD28	C25	.257	128.5	
	SD94	C27	.320	160.0	
	SE1	C31	.029	14.5	
	SE53	C31	.036	18.0	
	SE7	C31	.061	30.5	
		ACO - SHAM			
PROESTRUS	SD19	C22	.220	110.0	
	SD20	C23	.000	0.0	
	SD61	C25	.000	0.0	
	SD116	C27	.252	126.0	
	SE46	C31	.036	18.0	
	SD49	C24	.280	140.0	
ESTRUS - AM	SD58	C22	.180	90.0	
	SD30	C22	.062	31.0	
	SD30 & 26	C22	.365	182.5	
	SD90	C24	.049	24.5	
	SD67	C25	.000	0.0	
	SE40	C31	.000	0.0	
	SE5	C31	.040	20.0	
	SD42	C22	.089	44.5	
	SD26	C23	.125	62.5	
ESTRUS - PM	SD41	C24	.054	27.0	
	SD81	C24	.000	0.0	
	SD65	C24	.160	80.0	
	SD40	C24	.067	33.5	
	SD99	C25	.052	26.0	
	SD5	C25	.087	43.5	
	SD109 & 5	C25	.000	0.0	
	SD77	C25	.147	73.5	
	SD109	C25	.042	21.0	
	SE12	C28	.074	37.0	
	SE27	C31	.000	0.0	
	SE11	C28	.099	49.5	

Legend Same as APPENDIX VI, Page 149

(continued)

	Animal No.	Assay No.	Plasma LH µg/2 ml	Plasma LH ng/ml
		ACO - LESION		
PROESTRUS	SD10	C22	.160	80.0
	SD64	C24	.056	28.0
	SD53	C25	.232	116.0
	LG53	C27	.026	13.0
	SD105	C27	.000	0.0
	LG53 & SD105	C27	.058	29.0
	SE59	C31	.320	160.0
	SD6	C21	.083	41.5
	SE31	C28	.000	0.0
	SE26	C28	.034	17.0
	SD22	C23	.084	42.0
ESTRUS - AM	SD32	C22	.000	0.0
	SD2	C22	.061	30.5
	SD98	C24	.000	0.0
	SD7	C24	.000	0.0
	SD95	C25	.142	71.0
	SD59	C27	.041	20.5
	SE6	C28	.043	21.5
	SE51	C28	.000	0.0
	SE6,21,51	C28	.038	19.0
ESTRUS - PM	SD9	C22	.000	0.0
	SD11	C22	.000	0.0
	SD80	C24	.143	71.5
	SD50	C24	.104	52.0
	SD113	C27	.034	17.0
	SD120	C27	.084	42.0
	SE10	C31	.000	0.0
	SE10 & 2	C31	.000	0.0
	SE13	C31	.063	31.5
	SE16	C28	.000	0.0
		ST - SHAM		
ESTRUS - AM	SD108	C25	.000	0.0
	SD13	C25	.000	0.0
	SD14	C25	.000	0.0
	SD44	C25	.066	33.0
	SD84	C25	.000	0.0
	SD104	C25	.000	0.0
	SE44	D28	.067	33.5
	SE17	D28	.110	55.0
	SE17 & 44	D28	.061	30.5
		ST - LESION		
PROESTRUS	SD21	C22	.049	24.5
	SD78	C24	.000	0.0
	SD97	C24	.140	70.0
	SD73	C24	.202	101.0
	SD56	C25	.140	70.0
ESTRUS - AM	SD100	C24	.054	27.0
	SD33	C24	.153	76.5
	SD96	C25	.055	27.5
ESTRUS - PM	SD46	C24	.099	49.5
	SD66	C24	.093	46.5
	SD83	C25	.000	0.0
	SD12	C27	.051	25.5

Legend Same as APPENDIX VI, Page 149

(continued)

	Animal No.	Assay No.	Plasma LH µg/2 ml	Plasma LH ng/ml
		AL - SHAM		
PROESTRUS	SE4	C31	.044	22.0
	SG16	D18	.000	0.0
	SG16	D18	.000	0.0
	SG8	D18	.044	22.0
	SG10	D18	.000	0.0
	SG8 & 10	D18	.000	0.0
	SG17	D18	.218	109.0‡
	SG8 & 17	D18	.044	22.0
ESTRUS - AM	SE43	C31	.049	24.5
	SE19	C31	.000	0.0
	SE19 & 35	C31	.047	23.5
	SG13	D18	.064	32.0
	SG9	D18	.000	0.0
	SG9 & 13	D18	.000	0.0
	SG11	D18	.000	0.0
	SG11	D18	.042	21.0
	SG21	D18	.054	27.0
	SG13 & 21	D18	.000	0.0
		AL - LESION		
PROESTRUS	SE28 & 36	C31	.059	29.5
	SE49	C31	.080	40.0
	SE54	C31	.020	10.0
	SG6	D18	.088	44.0
	SG18	D18	.000	0.0
	SG6 & 18	D18	.000	0.0
ESTRUS - AM	SE19	C31	.000	0.0
	SE19 & 35	C31	.047	23.5
		4-MONTH STUDY: CONTROL		
PROESTRUS	SF3	D28	.000	0.0
	SF2	D28	.000	0.0
	SF2	D28	.000	0.0
	SF19	D28	.000	0.0
	SF3 & 19	D28	.113	56.5
	SA11	B41	.041	20.5
	SA24	B37	.312	156.0‡
ESTRUS - PM	SF7	D28	.000	0.0
	SF7	D28	.000	0.0
	SF8	D28	.000	0.0
	SF17	D28	.000	0.0
	SA7	B43	.000	0.0
	SA15	B41	.049	24.5‡
		ACO - SHAM		
PROESTRUS	SA14	B47	.255	127.5
	SA23	B47	.050	25.0
	SA28	B47	.098	49.0
ESTRUS - PM	SF12	D28	.000	0.0
	SF12 & 14	D28	.000	0.0
	SF22	D28	.000	0.0
	SF22	D28	.000	0.0
	SF14	D28	.000	0.0
	SF11	D28	.000	0.0
	SF11 & 14	D28	.000	0.0
		ACO - LESION		
PROESTRUS	SA29	B47	.490	245.0
	SA33	B47	.270	135.0
	SA19	B41	.062	31.0
ESTRUS - PM	SF46	D28	.104	52.0
	SF5	D28	.540	270.0‡
	SF18	D28	.000	0.0
	SF16	D28	.000	0.0
	SF16, 5, 46	D28	.000	0.0
	SA3	B43	.101	50.5
	SA1	B35	.035	17.5
	SF23	D28	.000	0.0
	SF23	D28	.000	0.0
	SF23	D28	.000	0.0

Legend Same as APPENDIX VI, Page 149 (continued)

	Animal No.	Assay No.	Plasma LH µg/2 ml	Plasma LH ng/ml
		ACO - LESION		
ESTRUS - AM	SA27	B47	.033	16.5
		POA - SHAM		
ESTRUS - AM	SE39	C49	.037	18.5
	SE50	C49	.048	24.0
		POA - LESION		
ESTRUS - AM	SE37	C49	.000	0.0
	SE45	C49	.142	72.0
		IC - LESION		
ESTRUS - AM	SA21	B47	.000	0.0
		HIPP - LESION		
ESTRUS - AM	SA17	B41	.000	0.0
		DMNT - LESION		
ESTRUS - AM	SA20	B41	.128	64.0

Legend Same as APPENDIX VI, Page 149

	RAT NO.	ASSAY NO.	P*	95% CL†	λ
		CONTROL			
PROESTRUS	LG1	C13	24.27	15.52 - 40.03	.202
	SB38	C6	22.13	14.07 - 34.21	.192
	SB12	C5	28.91	17.91 - 50.96	.218
ESTRUS - AM	SH61	D29	10.05	6.26 - 17.60	.216
	LG19 LG80	C34	8.70	6.58 - 11.58	.127
	SH27	D12	6.55	3.77 - 10.76	.222
ESTRUS - PM	SI20 SI9	D12	8.40	5.20 - 13.76	.210
	SH58 SH7	D27	11.18	7.73 - 17.01	.169
	SB7 SB21	C2	7.9	4.35 - 14.29	.250
METESTRUS	SH5	D10	11.83	8.04 - 17.58	.172
	SF4 SF41	C44	11.80	7.11 - 22.08	.231
	SF25 SF35	C44	11.70	5.90 - 25.86	.296
	SF38	C48	*16.77	9.92 - 32.38	.239
	SE62	C20	*11.41	8.05 - 16.96	.160
		ACO - SHAM			
PROESTRUS	SH57	D27	15.55	11.27 - 21.02	.137
	SB10	C10	15.90	9.55 - 24.43	.196
	SB9	C12	23.84	17.60 - 32.80	.138
ESTRUS - AM	SI60	D34	8.93	6.63 - 11.79	.127
	SI56	D30	8.43	5.11 - 13.08	.200
	SH34	D20	9.74	7.22 - 12.85	.127
	SI40 SI45	D32	*13.61	8.88 - 22.84	.194
ESTRUS - PM	SB34	D15	15.75	10.45 - 24.67	.184
	SB16	C10	11.56	7.77 - 16.94	.171
	SB37	C1	13.57	9.81 - 18.66	.143
METESTRUS	SH48	D32	14.75	10.88 - 20.55	.138
	SB15	C5	12.66	7.94 - 20.16	.202
	SB40	C14	10.20	5.06 - 18.63	.267
	SH13	D29	**11.04	4.26 - 28.30	.358
		ACO - LESION			
PROESTRUS	SI44	D12	24.51	13.18 - 45.95	.261
	SI29	D20	19.44	13.85 - 26.68	.144
	SB42	C14	25.80	17.20 - 40.40	.185
	SB27	C10	*15.06	10.72 - 20.10	.129
ESTRUS - AM	SI1	D33	11.67	8.30 - 16.22	.149
	SH12	D32	11.22	7.68 - 16.09	.161
	SH18	D30	13.32	9.28 - 19.59	.164
ESTRUS - PM	SI28	D34	17.89	13.12 - 24.44	.139
	SI33	D32	24.50	14.43 - 48.07	.243
	SI31	D30	15.37	10.21 - 22.98	.178
	SB25	C2	15.50	10.91 - 22.22	.158
	SH54	D29	*16.22	4.51 - 59.74	.433
METESTRUS	SB35	C2	17.05	12.05 - 45.07	.262
	SB26	C5	20.09	14.40 - 28.84	.152
	SH42	D30	15.36	12.00 - 19.41	.107
	SI24	*D29	10.54	5.47 - 17.17	.223

P*Mean Potency [illegible] 95% Confidence Limits Calculated from the Assay

*Pituitary Potency Rejected Because of Non-Parallelism between the Standard LH and Pituitary Dilutions

**Pituitary Potency Rejected Because $\lambda > .300$

λThe Index of Precision, Variance ÷ Slope

†95% Confidence Limits Calculated from the Assay

(continued)

	RAT NO.	ASSAY NO.	P*	95% CL	λ
			CONTROL		
PROESTRUS	SD48	C43	20.64	14.74 - 28.62	.148
	SD17	C48	24.78	18.42 - 33.66	.135
	SD4	C40	24.82	15.24 - 43.38	.220
ESTRUS - AM	LG38	D5	10.37	5.37 - 17.83	.245
	SD74	D3	16.77	12.01 - 23.84	.152
	SD60	C48	10.95	7.29 - 15.80	.167
	SD23	C41	*34.90	22.44 - 64.92	.192
ESTRUS - PM	SD28	C52	16.88	9.76 - 29.69	.236
	SD94	C45	12.31	7.82 - 18.21	.179
	SD55	C42	18.27	13.67 - 24.33	.129
	SD71 SD36	C38	*18.31	12.79 - 29.15	.156
			ACO - SHAM		
PROESTRUS	SD22	C52	18.93	11.69 - 29.54	.200
	SD80	C43	26.90	20.60 - 35.63	.122
	SD69	D1	17.32	11.47 - 25.72	.178
	SD20	C44	**20.46	8.48 - 46.14	.330
ESTRUS - AM	SD67	D10	13.15	8.10 - 21.50	.211
	SD90	D9	10.31	5.41 - 17.15	.231
	SD26	D1	15.97	10.20 - 24.50	.191
	SD58	D3	*27.25	18.10 - 45.40	.187
	SD30	D5	*7.49	2.12 - 14.84	.308
ESTRUS - PM	SD40	C52	13.95	7.59 - 23.77	.238
	SD65	C50	17.32	11.30 - 26.46	.186
	SD41	C45	17.86	13.13 - 24.34	.138
			ST - LESION		
PROESTRUS	SD97	D26	14.30	8.80 - 21.87	.194
	SD21	D24	12.47	7.19 - 19.53	.206
	SD78	D22	19.06	13.33 - 27.56	.161
ESTRUS - AM	SD35	D22	11.84	8.00 - 17.04	.165
	SD33	D24	10.62	6.73 - 16.16	.190
	SD100	D24	13.02	8.07 - 21.07	.208
	SD96	D26	*5.45	2.80 - 8.69	.206
ESTRUS - PM	SD46	D24	23.83	13.79 - 49.60	.251
	SD66	D22	12.54	8.30 - 18.25	.171
	SD12	D27	17.91	13.44 - 24.60	.131
	SD83	D26	10.15	6.35 - 14.85	.173
			ACO - LESION		
PROESTRUS	SD105	D12	22.03	14.29 - 35.60	.196
	SD64	C45	13.34	8.21 - 20.18	.189
	SD6	C48	17.87	13.87 - 23.19	.115
	SD10	C42	18.55	13.83 - 24.48	.127
ESTRUS - AM	SD95	D10	16.82	9.31 - 32.30	.257
	SD2	D1	13.97	9.61 - 19.50	.157
	SD98	D3	13.79	9.39 - 20.14	.164
	SD7	D5	*4.77	0.53 - 11.34	.390
	SE35	D26	*15.39	10.35 - 23.65	.180
	SE14	D22	*7.94	4.69 - 12.01	.187
ESTRUS - PM	SD120	D5	15.23	7.84 - 27.03	.255
	SD9	C51	22.36	16.04 - 31.44	.150
	SD80	D1	14.96	9.12 - 23.20	.203
	SD11	C45	20.45	13.76 - 31.19	.179
	SD113	D3	*26.67	19.62 - 37.84	.140
	SD50	C52	*13.65	7.59 - 21.69	.212

Legend Same as APPENDIX VII, Page 155 (continued)

APPENDIX VII: INDIVIDUAL PITUITARY LH POTENCIES 4-MONTH STUDY (continued)

	RAT NO.	ASSAY NO.	P*	95% CL	λ
			CONTROL		
PROESTRUS	SF3	D34	19.14	15.52 - 23.44	.093
	SA11	C34	12.81	5.62 - 27.08	.300
	SA59	C37	8.21	4.89 - 12.83	.204
	SF2	D31	*26.08	17.66 - 43.83	.170
	SA24	C33	*11.76	5.97 - 19.47	.231
ESTRUS - AM	SA16	C39	10.12	7.21 - 13.85	.144
ESTRUS - PM	SF7	D33	16.68	11.46 - 25.29	.171
	SF17	D31	11.02	5.20 - 19.88	.267
	SA22	C33	15.51	10.11 - 24.74	.193
	SA15, SA7	C20	*15.39	8.58 - 36.55	.267
METESTRUS	SA2	C33	14.20	9.10 - 22.66	.197
			ACO - SHAM		
PROESTRUS - AM	SA6	C39	21.88	16.17 - 29.53	.135
PROESTRUS - PM	SA33	C37	13.92	9.71 - 19.59	.155
	SA14	C33	17.54	12.14 - 24.79	.157
	SA28	D15	*29.04	21.06 - 41.96	.146
	SA54	D9	*12.77	7.34 - 19.90	.201
ESTRUS - AM	SA34	C39	14.00	8.67 - 22.47	.206
ESTRUS - PM	SF12	D33	15.41	9.88 - 23.85	.192
	SF11	D31	16.76	11.00 - 25.75	.186
	SA50	C37	15.29	10.07 - 23.04	.182
			ACO - LESION		
PROESTRUS - PM	SA29	C34	17.10	12.66 - 23.25	.135
	SA38	C39	22.97	15.06 - 37.70	.193
	SA19	C20	*16.34	10.64 - 23.66	.169
ESTRUS - AM	SA56	D9	*21.15	15.35 - 31.20	.143
	SA48	D9	*18.67	14.39 - 25.12	.117
	SA27	D15	*19.62	13.36 - 31.56	.174
ESTRUS - PM	SF23	D31	19.42	11.28 - 34.53	.237
	SF5	D34	15.33	10.42 - 21.87	.162
	SA3	C34	17.39	9.87 - 31.54	.244
	SA60	C37	11.15	7.16 - 16.36	.175
	SF46	D33	*24.17	16.49 - 37.91	.175
	SA55	D15	*24.25	17.09 - 36.40	.159

Legend Same as APPENDIX VII, Page 155

APPENDIX VIII: INDIVIDUAL DATA FOR THE OVARIAN COMPENSATORY HYPERTROPHY STUDY

RAT NO.	LEFT OVARY WET WEIGHT (mg)	RIGHT OVARY WET WEIGHT (mg)	% OCH	CYCLIC STAGE AT AUTOPSY	EGGS*
		CONTROL - 0 DAYS OCH			
LG62	35.8	36.2	1.12	Proestrus	--
LG64	35.5	30.8	-13.24	Proestrus	--
LG65	38.0	36.6	-3.68	Proestrus	--
LG68	36.0	41.4	15.00	Proestrus	--
LG69	31.7	35.8	12.93	Proestrus	--
LG74	33.3	34.0	2.10	Proestrus	--
LG75	40.3	48.6	20.60	Proestrus	--
LG77	32.4	36.1	11.42	Proestrus	--
LG78	22.2	28.7	29.28	Proestrus	--
LG81	37.4	41.2	10.16	Proestrus	--
LG88	46.8	39.6	-15.38	Proestrus	--
LG101	37.3	37.3	0.00	Proestrus	--
LG67	37.6	30.2	-19.68	Proestrus	--
LG86	34.4	32.0	-6.98	Proestrus	--
LG87	34.0	42.1	23.82	Proestrus	--
LG94	37.2	34.2	-8.06	Proestrus	--
LH6	44.8	59.0	31.70	Proestrus	--
SF28	28.4	46.2	62.68	Metestrus	--
SF29	42.4	38.8	-8.49	Diestrus	--
SF30	45.2	39.0	13.72	Proestrus	--
SF31	56.2	51.0	-9.25	Estrus	--
SF32	49.4	43.2	-12.55	Diestrus	--
SF36	63.2	50.2	-20.56	Diestrus	--
SH10	37.3	28.5	-23.59	Metestrus	--
SH14	24.1	25.7	6.63	Estrus	--
SH43	24.5	25.7	4.89	Estrus	--
SH47	34.8	31.8	-8.62	Proestrus	--
		CONTROL - 10 DAYS OCH			
SD111	35.7	54.9	53.78	Diestrus	--
SD72	47.0	61.2	30.21	Diestrus	--
SD121	33.1	54.7	65.26	Diestrus	--
SD47	47.9	57.0	19.00	Metestrus	--
SD43	38.8	53.5	37.89	PSP	--
SD16	37.7	47.7	26.53	Metestrus	--
SD91	29.2	52.0	78.08	Metestrus	--
SD106	47.8	60.1	25.73	Diestrus	--
SD93	40.2	59.5	48.01	Diestrus	--
SD110	38.1	56.2	47.51	Diestrus	--
SD122	49.1	62.1	26.48	Diestrus	--
SD29	53.8	55.2	2.60	Metestrus	--
SH9	44.4	57.0	28.37	PSP	--
SH24	33.4	42.3	25.51	Estrus	--
SH25	38.2	46.7	22.25	Proestrus	--
SH39	33.0	51.7	56.66	Proestrus	--
SI26	34.0	49.0	44.11	Metestrus	--
SI42	37.2	38.8	4.30	Diestrus	--

*If no figure is given, then the animal was not inspected for oviductal eggs.

PSP = Pseudopregnant

(continued)

(continued)

APPENDIX VIII: INDIVIDUAL DATA FOR THE OVARIAN COMPENSATORY HYPERTROPHY STUDY

RAT NO.	LEFT OVARY WET WEIGHT (mg)	RIGHT OVARY WET WEIGHT (mg)	% OCH	CYCLIC STAGE AT AUTOPSY	EGGS
		CONTROL - 15 DAYS OCH			
LG6	26.2	55.0	109.92	Proestrus	--
LG16	48.4	87.1	79.96	Estrus	12
LG22	26.6	36.5	37.22	Metestrus	--
LG23	44.9	53.1	18.26	Proestrus	--
LG36	34.6	41.1	18.79	PSP	--
LG49	42.5	56.9	33.88	Diestrus	--
LG20	37.0	55.4	49.73	Diestrus	--
LG56	36.5	48.4	33.33	Proestrus	--
LG59	49.8	63.2	26.91	Proestrus	--
		CONTROL - 20 DAYS OCH			
SH2	40.1	47.8	19.20	Diestrus	--
SH16	30.2	57.3	89.73	Estrus	--
SH20	44.5	64.2	44.26	Metestrus	--
SH23	34.7	54.2	56.19	Estrus	--
SH30	39.8	65.1	63.56	Metestrus	--
SH31	40.7	70.3	72.72	Estrus	--
SH32	40.2	60.1	49.50	Metestrus	--
SH52	50.3	60.9	21.07	Diestrus	--
SH56	41.0	66.1	61.21	Estrus	--
SH59	35.6	49.1	37.92	Proestrus	--
		ST SHAM - 10 DAYS OCH			
LG46	33.4	49.1	47.01	Diestrus	--
LG52	46.8	42.4	-9.40	Proestrus	--
SD68	32.9	49.0	48.94	Diestrus	--
LG51	33.4	37.4	11.98	PSP	--
SG12	34.8	58.2	67.24	Diestrus	--
SG14	32.6	45.6	40.49	Diestrus	--
SG22	38.5	60.2	56.36	Metestrus	--
SG19	32.8	52.2	59.14	PSP	--
SG5	25.0	37.6	50.40	PSP	--
		ACO SHAM - 10 DAYS OCH			
SD112	31.0	47.8	54.19	PSP	--
SD85	30.6	43.0	24.27	PSP	--
SD123	29.2	42.1	44.18	PSP	--
SD102	51.0	59.0	15.69	Diestrus	--
LG57	41.1	49.8	21.17	Proestrus	--
SF39	37.5	51.0	36.00	Diestrus	--
SF48	36.1	50.7	40.44	Diestrus	--
SF9	37.0	45.6	23.24	PSP	--
SF42	42.2	57.7	36.73	Diestrus	--
SG5	25.0	37.6	50.40	PSP	--

Legend Same as APPENDIX VIII, Page 158

(continued)

(continued)

APPENDIX VIII: INDIVIDUAL DATA FOR THE OVARIAN COMPENSATORY HYPERTROPHY STUDY

RAT NO.	LEFT OVARY WET WEIGHT (mg)	RIGHT OVARY WET WEIGHT (mg)	% OCH	CYCLIC STAGE AT AUTOPSY	EGGS
		ST LESION - 10 DAYS OCH			
SD54	47.2	40.8	-13.55	PSP	--
SD124	38.0	36.9	-2.89	PSP	--
SG3	42.1	46.7	+10.90	PSP	--
SG20	33.3	39.4	+18.31	Estrus	--
SG2	38.9	38.6	-0.77	PSP	--
SG1	35.0	35.8	+2.30	PSP	--
		ACO LESION - 10 DAYS OCH			
SD119	46.3	58.4	+26.13	Estrus	13
SD89	42.3	45.2	+6.85	Metestrus	--
SD75	33.2	35.2	+6.02	PSP	--
SF15	59.3	49.1	-17.20	PSP	--
SF20	50.0	56.8	+13.60	Metestrus	--
SF33	47.9	54.1	+12.94	PSP	--
SF45	49.0	53.3	+8.78	Estrus	--
SF13	52.1	39.0	-25.14	PSP	--
		CEREBRAL CORTEX LESION - 10 DAYS OCH			
SI15	29.8	40.0	34.22	Metestrus	--
SI19	31.4	32.4	3.18	PSP	--
SI27	25.2	43.5	72.62	Metestrus	--
SI30	32.8	38.2	16.46	PSP	--
SI48	24.6	32.9	33.73	PSP	--
SI55	40.6	56.1	38.18	Estrus	-8
		ST LESIONS MISPLACED			
SD92	33.3	45.6	36.93	Metestrus	--
SI58	33.8	43.6	28.99	PSP	--
		ACO LESIONS MISPLACED			
LG2	31.1	41.9	31.73	PSP	--

Legend Same as APPENDIX VIII, Page 158

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APPROVAL SHEET

The dissertation submitted by Stuart W. Smith has been read and approved by the following five members of the dissertation committee.

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The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that necessary changes have been incorporated, and that the dissertation is now given final approval with reference to content, form and mechanical accuracy.

The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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