EFFECTS OF UNILATERAL KNIFE CUTS PLACED IN THE MEDIAL BASAL HYPOTHALAMUS ON SEVERAL HYPOTHALAMICALLY-MEDIATED FUNCTIONS IN THE FEMALE RAT

by

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INTRODUCTION

The role of the central nervous system as a regulator of the pituitary gland is well established. The hypothalamus is of paramount importance in this regard, exerting its control over both the anterior and posterior pituitary gland. However, neural control of the adenohypophysis, unlike the posterior pituitary, is by vascular means, there being no significant neural connections between the anterior pituitary and the hypothalamus (100). This control is mediated by hypothalamic neurohormones, called releasing factors, secreted from neurons, presumably located for the most part in the ventral hypothalamus, into the primary capillary plexus of the hypophysial portal vessels to influence adenohypophysial secretions (33). Higher neural centers affect adenohypophysial tropic hormone secretion indirectly by acting through the ventral hypothalamus.

The real importance of the medial basal hypothalamus (MBH) was elucidated several years ago. This small region, which includes the arcuate and ventromedial nuclei, the medial part of the retrochiasmatic region and the median eminence, can maintain the structure and function of locally transplanted pituitaries (92) and, on this basis, was identified as the hypophysiotropic area (HTA). From experiments on animals subjected to surgical isolation of the MBH, Halász concluded that (a) basal secretion of adenohypophysial hormones is

largely maintained by the MBH; (b) this area represents a separate level in the hypothalamic control of the adenohypophysis; (c) the region is influenced by hormonal blood levels through feedback loops; (d) the region cannot maintain completely normal anterior lobe function; and (e) the normality of this function is maintained by a second hypothalamic or extrahypothalamic control level acting through the MBH to modify anterior lobe secretions (89).

Although neural control of anterior pituitary hormones is modulated by other hypothalamic and extrahypothalamic inputs, the MBH is recognized as a final neural pathway for regulating adenohypophysial secretion. The present studies were designed to explore the question as to whether there is anatomical and physiological redundancy in the bilateral arrangement of structures that reside in the MBH, more specifically, to determine whether the connections and neural elements which are present bilaterally in the MBH are essential for normal neuroendocrine regulation. The particular aim of these experiments was to assess the effects of hemiablation and hemiisolation of MBH on several endocrine control systems. The effects of these surgical manipulations on luteinizing hormone (LH), growth hormone (GH), and adrenocorticotropic hormone (ACTH) were studied under both non-stress or resting conditions and in response to stressful stimuli. In addition, because MBH has been implicated in the control of food intake and obesity, these endpoints were also studied in animals

subjected to unilateral hypothalamic surgery.

To furnish a background for understanding these studies and their significance, a historical review of neural control of those anterior pituitary hormones studied herein and of the regulation of food intake is presented below.

Neurovascular Control of the Adenohypophysis

Elucidation of the role played by the hypothalamus in the control of the adenohypophysis came with investigation of the blood supply to this area. A major contribution to our concepts of neuroendocrine control mechanisms was Popa and Fielding's description of the hypophysial portal vessels (160). Importantly, however, these investigators concluded that blood flowed from the pituitary to the hypothalamus. Wislocki and King confirmed the anatomical findings but proposed that the direction of blood flow was from the hypothalamus to the pituitary (203). With the portal vessel anatomy established, Hinsey was the first to suggest that humoral agents are secreted into the portal vessels to stimulate secretion of the anterior pituitary hormones (112). Most investigation of this area lay dormant for almost a decade because of the controversy concerning the direction of blood flow in the portal vessels. Studying the neural and vascular connections of the pars distalis, Green and Harris in 1947 (82) found no direct innervation of the anterior pituitary and largely confirmed

the earlier work of Wislocki and King on the vascular supply. Green and Harris also reiterated earlier hypotheses by proposing that adenohypophysial secretion was controlled through neurovascular means involving the portal vessels. Harris, in reviewing literature up to 1948, clearly enunciated this hypothesis and called attention to the lack of innervation of the pars distalis (99). More credence was lent to these proposals when Green and Harris convincingly demonstrated that portal blood flow in the living rat was from the hypothalamus to the sinusoids of the pars distalis (83). Several years later, it was shown that the pars distalis receives blood from a second set of portal vessels (46). These vessels originate in a capillary plexus in the lower pituitary stalk and neurohypophysis and distribute blood to the anterior pituitary by short portal vessels. In the pars distalis of some animals, including the rat, the portal vessels are the only source of blood supply (46).

Any theories concerned with hypothalamic control of the pituitary had to take into account the results of pituitary stalk-section experiments, the results of which were variable (99). After Harris and Jacobsohn (101) demonstrated in the rat that the hypophysial portal vessels regenerate after simple stalk section, much of the preceding controversy was resolved. These investigators emphasized the importance of the portal system for normal anterior pituitary function.

The investigations developed at this time also explained why pituitary transplantation to sites distant from the sella turcica resulted in depressed hypophysial function and why pituitaries placed in contact with the median eminence functioned normally (102). Earlier experimenters had demonstrated the existence of close contact between nerve fibers of the median eminence and the primary plexus of capillaries (82, 99). Harris suggested that nerves of the hypothalamus secrete humoral agents into the capillaries of the primary plexus in the median eminence and into the portal vessels of the anterior pituitary to stimulate or inhibit its cells to secrete hormones (100). Thus, evidence obtained during the 1940's and 1950's strongly supported neurovascular control of the adenohypophysis, although the humoral agents responsible for this control had not been found.

The importance of the stalk and median eminence as sources of hypothalamic hormones was demonstrated when an ACTH-releasing substance was extracted and partially purified from hypothalamic tissue by Slusher and Roberts (182). Up to this time, neurohypophysial substances, chiefly vasopressin, had been regarded as the ACTH-releasing substance (139). The properties of the substance isolated by Slusher and Roberts were distinctly different from any of the posterior lobe hormones.

In recent years, other hypothalamic neurohormones, called releasing factors or inhibiting factors depending on their activity in the

pars distalis, have been isolated by physical and chemical methods and three, luteinizing hormone-releasing factor (LRF) (6, 178), TSH-releasing factor (TRF) (35, 149) and growth hormone-inhibiting factor (GIF or somatostatin) (25) have been identified chemically and synthesized. Using radioimmunoassay (RIA) and immunohistochemical methods, LRF has been localized to the arcuate nuclei (33), TRF is found diffusely throughout the hypothalamus (33), and GIF is located in preoptic area neurons (5). All three hormones are concentrated in the median eminence (33). The purification and characterization of growth hormone-releasing factor (GRF) have evoked controversy since isolated material shown to release GH by bioassay is not active in releasing GH in a variety of animals measured by specific RIA (see 23 for review). This discrepancy has not been explained. Despite these problems, indirect evidence for GRF exists. Electrical stiumulation of the VMN produces elevations in plasma GH levels measured by RIA in the rate (19, 133, 134, 135). The isolation and characterization of corticotropin-releasing factor (CRF) have not been accomplished, in part due to inadequacy of existing assays for CRF activity. However, several polypeptides with CRF-like activity have been isolated from both hypothalamic and neurohypophysial tissues (23, 88). To date, all of the fully-characterized hypothalamic hormones are small polypeptides, and it appears that they are released

from neurons originating in or projecting through the medial basal hypothalamus, and that the secretory rate of each anterior pituitary hormone is under the control of at least one hypothalamic inhibiting or releasing factor (23, 139).

Neuroendocrine Control of LH Secretion

While studies using the techniques of stalk section and pituitary transplantation suggested hypothalamic neurovascular control of ovulation and reproductive cycles (99, 100), Sawyer, Everett, Markee and colleagues made important contributions to the field with pharmacological methods. They were able to block ovulation, indicative of LH release, in the rabbit using Dibenamine, an adrenolytic agent, and atropine, an anticholinergic agent, administered at appropriate periods after copulation (174, 175). Reflex ovulators such as the rabbit ovulate in response to vaginal stimulation. The blockade of ovulation that was produced suggested that adrenergic and cholinergic components, thus neural elements, were involved in the control of LH secretion (174). Additional studies suggested that the cholinergic and adrenergic components were arranged in series and that the cholinergic elements preceded the adrenergic (175).

Extending these experiments to the rat meant using different methods. This species is a spontaneous ovulator and does not need coital stimulation for ovulation. Ovulation occurs once during every

estrous cycle. Sawyer et al. found that they could induce ovulation in the rat 48 hr after subcutaneous estrogen injections (173). By injecting Dibenamine or atropine at specified times after estrogen administration, ovulation was prevented; however, exogenous LH produced ovulation even in the presence of these blocking agents. Estrogen, therefore, appeared to act partially on the nervous system to induce ovulation. These findings extended to a spontaneous ovulator what had been demonstrated in a reflex ovulator. Importantly, it was subsequently observed that these same blocking drugs given to rats on a standard light schedule before 2 pm on proestrus resulted in near universal blockade of ovulation, whereas administering the drugs after 4 pm was without effect on the ovulation which occurred 10-11 hr subsequently (67). The interval between 2-4 pm on proestrus was designated the critical period (CP) for the neural events that are essential for the ovulatory release of LH (60). Nembutal also blocked ovulation if given before 2 pm on proestrus (64). Ovulation could be postponed 24 hr by giving this barbiturate before the CP. If injected with the same time restraints on the subsequent 2 days, ovulation was again blocked, only occurring 24 hr after the last drug administration (65). These results demonstrated a 24 hr periodicity in the activation of the neural mechanisms governing LH release.

In summarizing this work, it was proposed that both spontaneous and reflex ovulators use a neural mechanism, probably located in the

hypothalamus, which is responsible for the release of LH. Furthermore, it appeared that adrenergic and cholinergic components are involved and that ovarian sex steroids may facilitate LH release by lowering the threshold of the hypothalamus or of the pituitary (130). The difference between the mechanisms controlling ovulation in the rat and rabbit was that in the former the hypothalamus is activated to stimulate LH release by an inherent, rhythmic mechanism, whereas in the latter it is activated by stimulation received during coitus.

Most of this early work has been substantiated using sensitive radioimmunoassays to measure circulating LH. Pentobarbital given before the CP prevents the normal preovulatory surge of LH, blocking ovulation (85). Only about 14% of the LH surge, occurring during the CP, is needed for ovulation (85).

Hypothalamic lesions - Experimental hypothalamic damage was first shown to cause gonadal atrophy by Camus and Roussy (37) and Bailey and Bremer (16) using dogs. These experiments were criticized because it was not convincingly demonstrated that the pituitary was not concomitantly damaged by impaired blood supply or direct surgical trauma. Smith (185) first clearly demonstrated in rats that gonadal atrophy could be induced by hypothalamic lesions without damage to the pituitary. Furthermore, lesions involving the base of the brain, specifically the tuber cinereum, produced both gonadal atrophy and obesity (108).

Later, it was found that electrolytic lesions placed in hypothalami of female guinea pigs produced four types of disturbances in reproductive functions: (a) animals with open vaginae; (b) animals with closed vaginae; (c) animals with regular sex cycles; and (d) animals with irregular cycles (51, 52). Dey correlated these several effects with variations in hypothalamic damage (50). He found that bilateral rostral hypothalamic lesions resulted in marked ovarian follicular development without evidence of ovulation or luteinization in those animals with open vaginae. Animals manifesting closed vaginal membranes were found to have ovarian atrophy and these effects were associated with bilateral median eminence lesions. In the remaining groups, hypothalamic lesions were variable in size and location.

Hillarp (111) used the rat to investigate the location of the hypothalamic centers controlling gonadotropin secretion elucidated earlier by Dey in the guinea pig. He demonstrated the existence of a region controlling luteinization in the preoptic-anterior hypothalamic region. Analysis of the effects of lesions at various sites led him to propose the presence of a fiber system with diffuse origins in the preoptic area and which became more discrete as it projected toward the tuberal region of the hypothalamus. Destruction of the anterior region or of its fiber tract did not appear to result in total inhibition of LH secretion as evidenced by maintained vaginal cornification.

Later reports confirmed Hillarp's findings, and many investigators found that median eminence lesions consistently caused anestrus with varying degrees of ovarian atrophy, while more rostral lesions resulted in persistent estrus with follicular ovaries (70). It was concluded that constant vaginal estrus resulted from abolition of the ovulatory surge of LH secretion; basal secretion was apparently unaffected.

Taleisnik and McCann, using the ovarian ascorbic acid depletion assay for measuring LH, noted that pituitary LH content was decreased to 33% of normal in rats made constant estrus with rostral hypothalamic lesions. In contrast, only 15% of the normal pituitary LH was present in rats with median eminence lesions (190). This report was the first to use a bioassay for demonstrating that hypothalamic lesions affect pituitary LH content.

As a follow-up of earlier experiments that defined a hypophysiotropic area in the MBH that could maintain the structure and function of transplanted anterior pituitaries (92), Halász and Pupp studied the effects of surgical "deafferentation" of the MBH in reproductive and other functions (91). Using this simple but ingenious technique on rats, they found two effects on reproductive cycles. Some rats demonstrated polyfollicular ovaries and persistent vaginal estrus, while others had persistent corpus lutea and constant vaginal diestrus. Halász and Gorski later extended these studies using

partial or total deafferentations of the MBH and reached conclusions consistent with those presented earlier by Hillarp (111). They found that pathways reaching the median eminence from the anterior hypothalamus are responsible for ovulation and the cyclic release of LH and that the MBH can only maintain tonic secretion of LH (90). These findings were subsequently confirmed (36). Experiments using RIA for measuring LH have led to similar results (24).

Köves and Halász found that including the preoptic-anterior hypothalamic area in the "deafferented" island of tissue was compatible with continued ovulation and reproductive cycles in some rats, suggesting that this area can act autonomously to stimulate the MBH to release LRF (121). They also found that unilateral arched cuts in the retrochiasmatic area were compatible with ovulation, suggesting that one-half of the preoptico-tuberal pathway, which projects to the MBH, is sufficient to maintain cyclic gonadotropin function.

Hypothalamic stimulation - As evidence accumulated suggesting that the nervous system controls many pituitary functions, other methods were applied to this area of investigation. One such method was that of electrical stimulation. Marshall and Verney were the first to show that electrical stimuli applied either to the lumbar spinal cord or the head of a rabbit could induce ovulation, implying that neural stimuli act through the anterior pituitary to modify its function (132). Similar studies were performed in the rat (96, 97). Within

the rabbit hypothalamus, stimulation of the anterior area was more effective than other areas, suggesting a localized region related to the control of ovulation (103). Markee et al. produced ovulation by hypothalamic stimulation and found that comparable stimulation of the pituitary was without effect (131). Stimulation of the pituitary was effective in causing ovulation only when large amounts of current were used, and this effect was considered to reflect current spread. These findings were supported by those of Harris (98). These results of stimulation supported the view that the hypothalamus played a major role in the induction of ovulation in this reflex ovulator.

Electrical stimuli had been applied infrequently to spontaneous ovulators due to the difficulty of producing ovulation in this species with this method. Critchlow, however, first effectively induced ovulation in rats by electrical stimulation (43). He administered pentobarbital to proestrous rats before the CP and stimulated through bipolar electrodes located in the hypothalamus. Ovulation resulted from basal hypothalamic stimulation. Using this approach, Everett found the preoptic area more sensitive to electrical stimulation than adjacent regions, in spite of atropine and pentobarbital blockade (61).

Barraclough applied electrical stimulation techniques to the androgen-sterilized female rat and suggested that hypothalamic control of LH secretion involved two regions: (a) the preoptic-anterior hypothalamic area (POAHA), which is responsible for the cyclic

ovulatory surge of LH, and (b) the arcuate- ventromedial nuclei region (VMN), which acts to maintain basal secretion of LH (17). Under this concept, the POAHA nerve fibers act through the arcuate-VMN region via the preoptico-tuberal pathway to produce cyclic LH release and ovulation.

This proposed dual nature of hypothalamic control of LH secretion has been substantiated and refined. Mapping studies have shown that stimulation of the POAHA consistently results in ovulation (63), and the threshold to electrical or electrochemical stimulation is lowest in this area (193). Stimulation of the POAHA also results in an increase in plasma LH that is similar in temporal and quantitative aspects with the normal preovulatory surge of LH (38, 40, 117, 194).

Everett (62), noting a direct relation between the size of an irritative electrochemical preoptic lesion and its effectiveness in inducing ovulation, proposed a "point-to-point" relationship between the origins of preoptico-tuberal nerve fibers and their terminations in the median eminence. Theoretically, as more preoptic area fibers are activated, more of the arcuate-VMN complex is stimulated to release LRF into the portal blood, resulting in increased LH secretion. Support for this scheme has accumulated (40, 41, 66, 191), although it remains unresolved as to whether the preoptico-tuberal fibers innervate the median eminence bilaterally or ipsilaterally.

Tejasen and Everett (191), in characterizing the preoptico-tuberal pathway observed that unilateral transection in the frontal plane at the rostral or caudal suprachiasmatic level blocked the ovulation-inducing effect of electrochemical stimulation of the ipsilateral, but not contralateral, preoptic area. These findings suggest that only one-half of the preoptico-tuberal system is sufficient for ovulation. Unilateral medial preoptic area (MPOA) electrochemical stimulation in animals with midsaggital cuts from the MPOA to the mid-VMN was compatible with ovulation. Similarly, ipsilateral MPOA stimulation in rats with unilateral mid-VMN transection in the frontal plane also resulted in ovulation. On the basis of these findings, Tejasen and Everett also concluded that there is a partial crossover of fibers in the retrochiasmatic plane such that the preoptico-tuberal pathway is represented bilaterally in the MBH.

Cramer and Barraclough (41) were unable to confirm the above findings. They found that unilateral or bilateral transection in either the retrochiasmatic or anterior hypothalamic plane blocked LH release and ovulation induced by ipsilateral MPOA stimulation. Animals with contralateral suprachiasmatic or anterior hypothalamic transections released the same amounts of LH as did the control-stimulated animals. However, these findings reinforced the view that only 50% of the preoptic-tuberal system is adequate for ovulation. Furthermore, these investigators reasoned that ipsilateral mid-VMN

transections in the frontal plane which failed to prevent the effects of MPOA stimulation still left the rostral half of this complex connected to the MPOA. It is possible, therefore, this portion of VMN is sufficient to support a normal LH surge. In summary, Cramer and Barraclough were unable to demonstrate any crossover of fibers anterior to the arcuate-VMN complex, and they suggested a unilateral point-to-point relationship within the preoptic-tuberal system.

Role of extrahypothalamic structures - As it became clear that the hypothalamus exerts a controlling influence over gonadotropin hormone secretion, interest was focused on the role of its afferent connections in this regard. The ovulation-blocking drugs, atropine, Nembutal and morphine, were found to evoke EEG changes unique to the midbrain reticular substance (172), implicating this area in the control of ovulation. More support for this concept came from Critchlow who suppressed ovulation in proestrous rats with rostral midbrain lesions (42). It was shown, however, that the aforementioned drugs exerted their influence at a hypothalamic rather than midbrain level (171), and Pekary et al. (158) showed that normal gonadotropin function is not dependent on midbrain afferents as both mesencephalic lesions and electrical stimulation did not interfere with ovulation. The role of the midbrain in controlling gonadotropin secretion remains unresolved.

Investigation of the role played by the amygdala in regulating gonadotropin secretion has resulted in controversy. Bilateral ablation of the amygdala in rats resulted in testicular atrophy, indicating depressed gonadotropin secretion (206) and suggesting a stimulatory role for the amygdala. More evidence for this concept was supplied by stimulation experiments. Using persistent estrous rats, Bunn and Everett induced ovulation by electrochemical stimulation of the amygdala (34). Others have used stimulation of the medial amygdala and stria terminalis to induce ovulation in persistent estrous rats (195).

Evidence for an inhibitory action of the amygdala has also surfaced. Elwers and Critchlow found precocious ovarian stimulation in prepubertal female rats after bilateral medial amygdaloid (56) or stria terminalis (57) lesions. Lawton and Sawyer demonstrated increased pituitary and peripheral LH concentrations in ovariectomized rats with medial amygdaloid lesions (126), and electrolytic stimulation of the corticomedial amygdala produced delayed vaginal opening in immature female rats (44).

Velasco and Taleisnik reported data that may resolve some of the controversy regarding the amygdala and its role in ovulatory processes. In proestrous rats, bilateral section of the stria terminalis blocked the scheduled ovulation, but it failed to cause a chronic disturbance in ovulation or sexual function (197). Confirmation of these data have appeared (32). Thus, this brain area does not

appear essential for ovulation, but it may tonically facilitate the hypothalamus and thereby modulate gonadotropin secretion (197).

The hippocampus appears to be inhibitory and again modulatory in its effects on gonadotropin release. Stimulation of this structure suppresses ovulation (196), whereas its ablation is compatible with normal gonadotropin secretion and ovulation (32, 197).

Feedback - It has been known for several decades that ovarian steroids may exert inhibitory or facilitatory effects on gonadotropin secretion (see 69 for references). For many years, both of these effects were thought to occur by direct actions on the adenohypophysis (69). Recently, however, evidence has accumulated from a variety of approaches to implicate the brain in these feedback controls (see 207 for review). Current evidence (207) favors the view that feedback regulation of gonadtropin secretion may involve both hypothalamic and adenohypophysial sites of action.

Stress - Unlike the GH and ACTH control systems which respond consistently to stressful stimuli, the LH secretory response to noxious stimuli is variable. Many reports are contradictory on this point and differences in experimental design have complicated conclusions.

It appeared initially that LH was unaffected by stress because ether and bleeding were reportedly without effect on afternoon LH levels in proestrous rats (204). Furthermore, ether inhalation,

laparotomy, and blood sampling did not alter LH concentrations throughout the estrous cycle (150). Dunn et al. (55) and Ajika et al. (1) were the first to report a significant effect of stress on LH levels. The former group demonstrated significant elevations in plasma LH after ether inhalation in male rats. The latter investigators showed that ovariectomized rats exposed to ether and bleeding increase LH concentrations in plasma, and Nembutal blocks the response, suggesting that neural pathways are involved. Normal male rats show an initial increase in plasma LH levels, followed by a gradual decrease, in response to separate bleeding and 2 min ether inhalation (124).

It appears that the initial level of LH and the status of the gonads may be important to the stress response. Immobilization stress was without effect on LH concentrations in male rats and in diestrous and estrous females with low gonadotropin levels (59). However, in proestrous rats with high levels of LH, this stressor sharply reduced these levels and blocked the LH surge for the 2-hr duration of the sampling period. Others have shown unaltered LH levels in ovariectomized rats after bleeding,whereas diestrous and ovarian steroid-treated, ovariectomized rats showed increased LH levels in response to this stressor (181). More recently, male rats were reported to show increased LH levels in response to immobilization (123).

Summary - Present evidence strongly suggests that neural control of LH secretion involves two levels of control. The first level is located in the MBH and is concerned with the tonic release of gonadotropins and maintains basal levels of secretion. The second level is located in the POAHA and controls the cyclic release of gonadotropins responsible for ovulation. The POAHA is apparently autonomous in its activation of cyclic LH secretion. Neural impulses travel from this area to the MBH via the preoptico-tuberal pathway, where it appears to terminate bilaterally. It seems that only one-half of this pathway is necessary for normal ovulation. From the MBH, the tuberoinfundibular tract projects to the median eminence where its terminals end on portal vessels. LRF is released from these terminals, causing both LH and FSH release. Extrahypothalamic structures influence this system by ultimately acting through the MBH. As with other hormonal systems, feedback loops exist to adjust adenohypophysial secretions. For LH, this feedback control appears to include both positive and negative components which exert effects at the anterior hypothalamic and adenohypophysial levels. It appears that LH is affected by a variety of stressors and that the initial level of LH and the status of the gonads may be important to the response.

Neuroendocrine Control of GH Secretion

Investigation of the control of GH secretion lagged behind that of other adenohypophysial hormones because of the lack of a specific target tissue and of an appropriate and sensitive assay. Early studies of pituitary transplantation had shown as ancillary findings that when the gland was transplanted to a site distant from the severed stalk, depressed growth generally was manifest (100). Other experiments demonstrated diminished growth when pituitary implants were placed so as to exclude vascularization from the median eminence, suggesting that the hypothalamus contained a mechanism which controlled growth (104, 188). Attempting to localize this mechanism, Halász noticed that anterior pituitaries grafted into the MBH of hypophysectomized immature rats produced significantly greater rates of body growth than if they were located outside this area (95). This finding suggested that the MBH was importantly involved in controlling GH secretion.

Hypothalamic lesions - Much of the information which bears on the neural mechanisms involved in the control of GH secretion has come from observing the effects of selective destruction of various brain areas. Reports of early experiments investigating hypothalamic control of food intake by the placement of VMN lesions included observations of depressed growth as incidental findings in some

animals (110). However, bilateral VMN lesions usually resulted in multiple endocrine deficiencies and variations in food intake that could have accounted for the retarded somatic growth (119). Hinton and Stevenson electrolytically destroyed the supraoptic area in young male rats and noted growth deficits unaccompanied by significant visceral or endocrine gland atrophy (113). Indirect evidence for a hypothalamic control of GH secretion came with the observation that insulin sensitivity which resulted from hypothalamic lesions could be corrected by GH (187). Reichlin found that massive lesions of the median eminence, primary portal plexus or pituitary stalk resulted in growth suppression in rats (164) despite replacement therapy to correct endocrine deficiencies and diet control (163). These lesions were accompanied by a reduction of pituitary GH content to 15% of normal (165).

Many of the above experiments involved lesions that were large and involved both neural structures and elements of the hypophysial portal system. Therefore, it was difficult to determine whether deficits reflected brain damage or were based on pituitary infarction. The experiments of Bach et al. (15) and Hinton and Stevenson (114) histologically ruled out vascular damage involving the pituitary in their animals with anterior hypothalamic lesions and impaired growth, thus establishing hypothalamic involvement in GH release.

Although retarded growth resulted from electrolytic lesions placed in more than one region of the hypothalamus (15, 113, 114, 163, 164), attempts were made to localize the area responsible. Destruction of the paraventricular nuclear region in kittens caused stunting (15) and a decreased number of acidophilic cells in the adenohypophysis; these cells are known to elaborate GH (see 20 for references). Bernardis and Skelton demonstrated that bilateral electrolytic lesions of the VMN in rats of various ages and both sexes consistently depressed growth and reduced pituitary acidophil counts (20, 21).

As with other endocrine systems, rapid advances were made with the advent of RIA for GH. Frohman and Bernardis were the first to use this method to show that VMN lesions placed in weanling rats result in low levels of pituitary and plasma GH (73). Demonstrating decreases in pituitary and plasma RIA-GH levels, linear growth and lean body mass in weanling rats with progressively larger VMN lesions, Bernardis and Frohman convincingly supported the suggestion that the VMN is the principal hypothalamic locus for control of GH secretion (18). More support was lent to these findings with the observation that VMN destruction produced with gold thioglucose resulted in depressed pituitary and plasma RIA-GH levels (148). Halász et al. isolated the MBH from the rest of the brain and observed the maintenance of basal GH secretion, implying that the MBH

is intrinsically capable of producing and releasing sufficient hypothalamic factor to maintain normal GH secretion (93).

The above results imply a stimulatory role for the hypothalamus in GH secretion. Deafferentation of the MBH, however, has resulted in increased growth rates and high levels of plasma GH in rats (156), and anterior hypothalamic lesions in squirrel monkeys have been associated with increased GH responses to stress (31). Such findings suggest the existence of neural elements that are inhibitory to the secretion of GH. Whether such inhibition is mediated through suppression of GRF and/or facilitation of GIF is not yet clear.

Hypothalamic stimulation - Although stimulation techniques have proved effective in studying neural control of several adenohypophysial hormones, this method has been applied to GH research only recently. Frohman et al. used electrical stimulation to complement their experiments with hypothalamic lesions which indicated that the VMN is an important locus for control of GH release (74). Stimulation of the VMN caused elevated plasma RIA-GH levels within 5 min, whereas cortical stimulation was without effect. Bernardis and Frohman subsequently achieved more precise localization when they obtained elevations in plasma RIA-GH levels only with stimulation applied to the VMN, its border zone or the median eminence (19).

Medial tuberal hypothalamic stimulation of the monkey produced similar effects on plasma GH concentrations (183). In a series of experiments, Martin's laboratory has largely confirmed the above work by demonstrating consistent plasma GH rises with arcuate-VMN stimuli; stimulation of other hypothalamic areas was ineffective in this regard (133, 134, 135).

Role of extrahypothalamic structures - As evidence accumulated to suggest hypothalamic regulation of GH secretion, the role of extra-diencephalic structures was also assessed in this respect. Partial or complete hypothalamic deafferentation which spared the VMN resulted in animals with increased linear growth (156). This finding raised the possibility of growth-inhibiting neural inputs to the MBH. Augmented GH responses were observed with ether stress in a few squirrel monkeys with lesions in the region of the optic chiasm, suggesting that structures in the preoptic region inhibit GH secretion (31). Mitchell et al. confirmed in the rat earlier reports of increased somatic growth resulting from hypothalamic cuts and concluded that forebrain structures, which normally inhibit growth, may do so through afferents that project to the MBH from the anterior direction (142). In this same report, normal to decreased plasma RIA-GH levels were observed 7 months postoperatively in animals with increased lengths. However, by studying GH levels more acutely after surgery, Mitchell et al. later found elevated plasma RIA-GH levels in MBH deafferented

rats at 7 and 11 weeks after surgery, during the period of accelerated growth (141). It was not determined whether the GH levels indicated increased secretion or deranged metabolism. Nevertheless, these findings extended previous reports by showing that the increased linear growth resulting from hypothalamic surgery may be associated with increased circulating levels of GH. Thus, afferents to MBH may inhibit both growth and GH secretion.

Evidence from a different approach has extended much of the above information. Electrical stimulation of the dorsal hippocampus, midbrain interpeduncular nucleus and basolateral amygdala caused increases in plasma RIA-GH levels, whereas decrements in GH levels were produced by stimulation of the corticomedial amygdala (133, 135) and preoptic region (133). It is interesting that the basolateral amygdala, which projects to the MBH by the ventral amygdalohypothalamic pathway, has a low threshold for the induction of GH release by electrical stimulation. In contrast, stimulation of the corticomedial amygdala, which connects with the MBH through the stria terminalis, inhibited GH release.

In addition to the above, other investigators (39) reported that non-stress plasma RIA-GH levels were significantly elevated in frontally-deafferented and significantly depressed in posterolaterally-deafferented rats. These findings again suggest that neural elements located outside the MBH, but acting through it, modulate the control

of GH secretion. According to their results, inhibitory influences reach the MBH through anterior connections, while facilitatory fibers project via posterior and lateral pathways.

Stress - With the development of a sensitive RIA for measuring GH, detailed studies of its regulation became possible for the first time. Investigation of the GH response to stress showed that a variety of noxious stimuli inhibited GH secretion in the rat (39, 53, 124, 142, 168, 177, 189). In contrast, primates demonstrate marked elevations of GH secretion in response to diverse stressors (23, 30). The basis for this interesting species difference has not been resolved. Nevertheless, it appears that the hypothalamus is the final pathway for mediation of the GH stress response in both primates and rats (30, 53). In the rat, the response to ether stress necessitates both an intact MBH (53) and connections from extrahypothalamic structures (142).

As indicated above, observations from deafferentation experiments have suggested that inhibitory GH pathways project to the MBH from the anterior direction (39, 142). Rice and Critchlow (168) recently found that ablation of the preoptic area actually caused reversal of the normal responses in rats so that stress produced increments in plasma GH levels. Ablation of the amygdala, hippocampus, septum or striatum was compatible with the normal stress response. These findings suggest that the preoptic area is an

important region in mediating the stress-induced inhibition of GH secretion and that the other telencephalic structures may not be essential for this response. Consistent with the above studies, Alpert et al. (5) found that somatostatinergic neurons are, in part localized to the preoptic area and Arimura et al. (14) discovered that the stress-induced inhibition of GH secretion is due, in part, to increased release of somatostatin.

Summary - It is well established that GH secretion is regulated by the nervous system and, in particular, by the hypothalamus. It appears that the regulation of GH secretion is under dual control. Studies involving a variety of approaches have implicated the MBH, specifically the arcuate and VMN, as the neural substrate responsible for stimulating GH release. Neurons from these nuclei project to the median eminence where GRF is presumably released into the pituitary portal circulation to stimulate pituitary GH secretion. Inhibition of GH secretion is thought to be mediated by GIF or somatostatin synthesized by neurons which appear to be located in the preoptic area. This hormone is also presumed to be released into the portal circulation, to affect GH secretion. Other extrahypothalamic structures affect GH secretion by acting through the MBH. Facilitatory influences may reach the MBH through posterolateral pathways. The rat differs markedly from primates in its GH response to stress. Whereas primates secrete GH in response to

stress, stress causes an inhibition of such secretion in the rat. The basis for this species difference has not been resolved. The rat's stress-induced inhibition of GH secretion appears to be in part mediated by somatostatin neurons located in the preoptic area.

Neuroendocrine Control of ACTH Secretion

Stress - Most of the studies elucidating the neural regulation of ACTH secretion have been concerned with factors regulating the increased secretion of ACTH under conditions of stress. Hume and Wittenstein (115) and DeGroot and Harris (48), working independently, were among the first to pursue the problem of hypothalamic regulation of pituitary-adrenal function. The data from these early studies suggested that the intact hypothalamus is essential for the normal secretion of ACTH in response to stress; that hypothalamic lesions, in the presence of an intact pituitary and adrenal cortex, abolish the stress response; that stimulation of the hypothalamus mimics a stress stimulus with regard to ACTH secretion; and that a hormonal link may mediate hypothalamic control of ACTH secretion. Harris (100), summarized the early experiments involving effects of hypothalamic lesions and stimulation on ACTH secretion and postulated that under basal, non-stress conditions ACTH secretion is maintained by an intrinsic hypothalamic drive mediated via the hypophysial portal vessels. Harris also suggested that during periods of stress, two

mechanisms are operant. The first involves neural stressors which act through neural pathways to activate hypothalamic secretion of a humoral mediator that traversed the hypophysial portal vessels to cause release of ACTH from the adenohypophysis. The second mechanism was presumed to involve stressors which act through the systemic circulation to induce ACTH secretion by actions on the hypothalamus and/or pituitary. As indicated below, these early suggestions appear essentially correct.

McCann studied the effect of hypothalamic lesions in the rat and confirmed that, as in other species, large median eminence lesions abolish the adrenal response to surgical stress without causing adrenal atrophy (138). He found, however, that destruction of the hypophysial portal vessels caused adrenal atrophy. Apparently, the pituitary could autonomously release sufficient ACTH to prevent adrenal atrophy under resting conditions. Many later experiments confirmed that large median eminence lesions block both the release of ACTH to a wide variety of acute stresses and prevent compensatory adrenal hypertrophy after unilateral adrenalectomy (75). Presumably, the neuronal terminals of the median eminence contain CRF which is released into the portal vessels to effect ACTH release (75).

Brodish (29) lesioned several areas of the hypothalamus and found delayed corticosterone responses to stress, suggesting that a large, diffuse hypothalamic area is concerned with control of ACTH

secretion. Snyder and D'Angelo obtained significant ACTH and corticosterone responses in the rat by stimulating the POAHA or the ventral hypothalamus (45, 186) and also concluded that widespread areas of the hypothalamus are responsible for the regulation of ACTH secretion. Further stimulation experiments have reinforced these conclusions (162).

Although the hypothalamic area concerned with ACTH release is diffuse, most of the CRF-producing neurons with terminals in the median eminence are believed to reside in the MBH. Halász et al. (94) gave support to this view by demonstrating that the MBH could be completely deafferented and still support ACTH secretion in response to certain stressors. In addition, these investigators showed that the surgically-isolated MBH is capable of maintaining basal ACTH secretion and adrenal compensatory hypertrophy. They concluded that there are two levels of organization of neural structures that control ACTH secretion. One level is located in the MBH, and it can independently maintain ACTH secretion under certain conditions. The second level consists of extrahypothalamic structures which act through the afferent connections to the MBH to affect other aspects of pituitary-adrenal activity.

Other investigators have also demonstrated that the surgically-isolated MBH-pituitary unit can chronically maintain non-stress levels of pituitary-adrenal function within the normal range and

support responses to a variety of stressful stimuli (3, 68, 84, 94, 155). In fact, it appears that responses to ether (136) and immobilization (169) persist in preparations with median eminence-pituitary islands, i.e., after removal of all forebrain tissue down to the level of the median eminence. Other stressors require intact neural connections with MBH for the induction of ACTH secretion. For example, tibia fracture activates the pituitary-adrenal system via pathways that enter the MBH from the anterolateral direction (4, 136).

Although retention of stress responses in rats with median eminence-pituitary islands is seemingly discrepant with previous experiments (48, 115) which demonstrated that large hypothalamic lesions block the response to ether, this is not necessarily the case. Responses to ether do not persist after chronic MBH ablation (54). Because all of the forebrain removal experiments cited above were performed approximately 24 hr after surgery, it is possible that the presence of stress responses in the acute but not the chronic situation reflects the transient presence of CRF-loaded terminals in the median eminence. This CRF may be releasable in response to blood-borne or other stimuli. With time, these terminals would degenerate and no CRF would be available. The above is conjecture, and it is clear that this phenomenon needs to be explored experimentally.

Because of their intimate connections with the hypothalamus, there has been considerable interest in the potential roles of the

midbrain and limbic system structures in controlling pituitary-adrenal function. The midbrain has been studied by lesion, stimulation and transection techniques. Some investigators have found that lesions in this area result in loss of stress-induced increases in corticosteroids (120), whereas others found no effect (129). The midbrain apparently contains elements that both activate and inhibit ACTH secretion (129). As illustrated by stimulation and ablation experiments, the amygdaloid complex may exert excitatory influences on ACTH secretion (3, 4, 162), whereas the hippocampus may be inhibitory (129, 176), even in humans (170). Limbic structures appear to be capable of influencing or "modulating" ACTH secretion, but they do not appear to be essential for the control of responses to most stressful stimuli.

Circadian rhythm - Although the hypothalamo-pituitary-adrenal unit has considerable autonomy in maintaining ACTH secretion in non-stress and some stress situations, circadian rhythmicity of pituitary-adrenal function appears to depend on intact extrahypothalamic connections. Both MBH deafferentation (94, 155) and forebrain removal (153) abolish normal circadian rhythmicity. More selective deafferentations or MBH lesions have shown that anterior projections to the MBH are essential for maintaining this circadian rhythm (94, 144, 155).

On the basis of a report by Moberg et al. (143), it appeared

that the hippocampo-fornix system might be important in controlling the circadian rhythm in pituitary-adrenal function. However, recent reports indicate that removal of hippocampus and/or fornix transection (200) are compatible with normal circadian rhythmicity. Ablation of septum which incidentally interrupted both the stria terminalis and fornix also failed to interrupt this rhythm (201). Therefore, it does not appear that these limbic structures are essential for this rhythm. In contrast, the region of the suprachiasmatic nucleus, through which retinohypothalamic connections pass (145), may be important in this regard (144).

Feedback - Since it was recognized that the hypothalamus plays a vital role in the neural regulation of adenohypophysial secretions, it has been assumed that it is also indispensable for feedback control of pituitary secretion. However, Ganong and Hume (76) were the first to show that the hypothalamus was not essential for negative feedback control of ACTH secretion. They lesioned the MBH in dogs and administered large amounts of cortisol to both experimental and control groups. Adrenal atrophy occurred in both groups. These authors concluded that this feedback effect was mediated through the pituitary.

A voluminous literature on this subject has developed. Mangili et al. (129) summarized much of this field and concluded that negative feedback control of ACTH secretion is exerted at a multiplicity of

sites, including the pituitary, hypothalamus and extrahypothalamic structures such as the hippocampus and midbrain reticular formation. Evidence was also presented suggesting a direct or short feedback loop by which ACTH inhibits its own secretion (129). Kendall, also reviewed the feedback literature and concluded that although the primary site of corticosteroid feedback is still unknown, the bulk of experimental evidence favors the pituitary as the major site of action (118).

Summary - The regulation of ACTH secretion appears to be mediated primarily by the hypothalamus. The hypothalamic area concerned with this control is diffuse as shown by both lesion and stimulation experiments. Extrahypothalamic structures and projections appear to be important for some pituitary-adrenal responses to stress, as in the response to tibia fracture, but not for responses to ether or immobilization. Extrahypothalamic structures are apparently essential for circadian rhythmicity of the pituitary-adrenal axis, and the POAHA seems most important in this regard. The hippocampus, fornix and septum do not appear to be essential for this rhythm. Negative feedback control of ACTH secretion may exist at several sites, including the pituitary, hypothalamus and/or the hippocampus.

Neural Control of Food Intake

Hypothalamic lesions - The role of the hypothalamus in the pathogenesis of certain kinds of experimental obesity has been extensively investigated both experimentally and clinically. Early clinicians noted the association between obesity and genital atrophy on the one hand and damage to the region of the pituitary on the other (72). Camus and Roussy concluded that lesions at the base of the brain produce this "adiposogenital syndrome" in experimental animals (37). Bailey and Bremer, using dogs (16) and Smith, using rats (185), demonstrated that lesions of the hypothalamus resulted in adiposity and suggested that this effect was not due to damage to the pituitary.

Controversy persisted regarding the respective roles played by the hypothalamus and the hypophysis in experimental obesity. In a brilliant set of experiments, Hetherington and colleagues demonstrated the importance of the hypothalamus and the non-essential role played by the hypophysis in the development of this form of obesity. Obesity did not occur in hypophysial-damaged animals unless concomitant hypothalamic damage occurred (105), and the marked obesity resulting from hypothalamic lesions was not affected by subsequent hypophysectomy (109). Furthermore, whereas chronic hypophysectomy did not result in obesity, subsequent placement of

hypothalamic lesions was followed by rapid weight gain (106). Using stereotaxic equipment to avoid hypophyseal damage, Hetherington and Ranson made bilateral electrolytic lesions in the ventral hypothalamus of rats and produced a high incidence of obesity, characterized mainly as stored lipid (108). Damage rostral and dorsal to the VMN was without effect, leading Hetherington to conclude that this type of obesity was secondary to destruction of nervous elements in or near the VMN (107). The efficacy of producing adiposity with stereotaxically-placed hypothalamic electrodes was confirmed by Tepperman et al. (192). Hetherington and Ranson also found that hypothalamic lesions involving the VMN were most effective in producing obesity, and that unilateral damage was ineffective (110). The adiposity resulting from hypothalamic damage appeared to be due to hyperphagia rather than to inactivity, inability to utilize stored lipids or changes in temperature regulation (27, 28).

While studying the effect of electrolytic lesions placed in other areas of the hypothalamus on food intake in rats, Anand and Brobeck noted that lateral hypothalamic lesions in the rostro-caudal plane of the VMN caused a complete cessation of eating that ended in the death of the animal several days postoperatively (9). These authors designated this lateral hypothalamic area as the "feeding center." Incidentally, these authors also reported that unilateral lateral hypothalamic ablation was without effect on food intake. Confirmation

of the above findings in cats and monkeys was soon furnished by Anand et al. (12). These investigators postulated the existence of dual hypothalamic centers regulating food intake involving the VMN, the ventrolateral area and the nerve fibers connecting these two neural centers. According to this view, the ventrolateral area represented the "feeding center" responsible for the initiation of feeding, while the VMN acted as the "satiety center" capable of exerting control over the lateral area. Lesions of the lateral hypothalamic area (LHA), however, had only a temporary effect on food intake. If lesioned animals were given temporary nutritive support, by stomach tube or parenteral alimentation, complete recovery often ensued (7).

One test of the proposed dual hypothalamic control of food intake involved studying the effects of unilateral lesions. As pointed out by Gold (80), any theory concerning the nature of the hypothalamic regulation of food intake must account for the relative ineffectiveness of unilateral VMN (110) or LHA (9) lesions in producing hyperphagia and obesity. Subsequently, Mayer and Barrnett found that unilateral VMN lesions resulted in animals with about 50% of the weight gain observed in bilaterally-lesioned animals (137). This finding of obesity occurring with unilateral lesions, was later confirmed in an abstract (198). Gold later produced small unilateral lesions in the ventrolateral area and noted transient aphagia (78). More recently,

Gray and Everett (81) observed hypophagia lasting up to 30 days after placement of a unilateral LHA lesion. Thus, there is some evidence that unilateral manipulations of the hypothalamus can affect food intake and obesity.

Further support for the "center" concept of food intake regulation came from a different approach. It was known that gold thioglucose (GTG) injections often caused obesity in mice (26). Bilateral VMN damage resulted from GTG injection, and it was found that GTG-obese mice closely resemble their electrolytic-lesioned counterparts with regard to hyperphagia, ratio of adipose tissue to lean body mass and location of hypothalamic injury (128).

However, the dual control theory of food intake has been questioned recently. Reynolds used both radio-frequency current and electrolysis to perform bilateral VMN destruction in rats (166). Hyperphagia was demonstrated only with the latter technique. This difference in results with the two types of lesions, he concluded, could occur only if an "irritative" hypothesis regarding food intake was adopted (167). Inherent in this hypothesis is the ability of deposited metallic ions, resulting from electrolytic but not radio-frequency lesions, to continuously stimulate the feeding center in the LHA so that the animal becomes hyperphagic. Thus, increased food intake would not result from simple elimination of a VMN "satiety center." Confirmation of these results has come from experiments

comparing the effectiveness of producing hyperphagia with irritative vs. non-irritative methods (161).

Although Reynold's hypothesis regarding the role of irritation in producing hypothalamic obesity has produced considerable controversy concerning the concept of dual hypothalamic mechanisms controlling feeding, several contradictory findings have appeared. Epstein procainized the VMN bilaterally through chronically implanted cannulae and observed feeding in sated rats (58). He also found that hypertonic saline, assumed to stimulate neurons, decreased eating in hungry rats when applied to VMN. These substances placed in the LHA produced the opposite effects on feeding. In addition, VMN lesions produced with radiofrequency current have produced obese hyperphagic rats (105). Other techniques which do not deposit iron such as suction ablation of the VMN (159) and knife cuts (2, 80, 116, 179) also cause hyperphagia and obesity. However, all lesion techniques are potentially irritative, so the issue raised by Reynolds is difficult to resolve.

When they proposed a VMN "satiety center" and a LHA "feeding center," Anand et al. suggested that the former inhibited the latter via fiber connections (9). This was largely based on the finding that animals made obese and hyperphagic by VMN lesions could be made aphagic with lateral hypothalamic lesions (9). These fibers were not identified until Arees and Mayer induced ventromedial region

lesions in GTG-injected mice and demonstrated degenerating fibers originating in the VMN and terminating in the lateral hypothalamic area (13). With the development of microsurgical techniques, several investigators performed transections between the VMN and LHA, leaving both nuclei intact (2, 80, 116, 179). Hyperphagia and obesity, similar to that produced by electrolytic lesions (116), result. Knife cuts along the anterior tip of the VMN are most effective (80). It appears that maximal obesity can be obtained with cuts that isolate the ventral half of VMN and adjacent arcuate nucleus from the remainder of the brain (156).

Although extensive research has partially elucidated the neural structures involved in regulating food intake, little is known about the afferent signals that regulate food intake. Several theories have been proposed, and as summarized by Anand (8), these proposals contend that there are specific receptors in the hypothalamus that detect alterations in circulating glucose, products of fat metabolism or amino acids or which measure the heat generated by metabolism of nutrients. No single theory has completely explained the experimental results. However, Panksepp (157) has proposed a new hypothesis which maintains that the medial hypothalamus contains neurons that monitor body nutrient stores and that these neurons are responsible for long-term regulation of feeding. This regulation is achieved by modulating the LHA via excitatory and inhibitory

influences, depending on whether body nutrient stores are low or high, respectively.

Hypothalamic stimulation - Electrical stimulation has also been used to study the role of the hypothalamus in the control of food intake. The results largely support the concepts that have been derived from the lesion approach. Delgado and Anand were the first to demonstrate a significant increase in food intake in cats following electrical stimulation of the lateral hypothalamus through permanently-implanted electrodes (49). Confirmation came from Larsson who showed a maximal hyperphagia in goats and sheep after electrical or chemical stimulation of the lateral hypothalamic nucleus (125). While attempting to map out areas in the hypothalamus effective in altering food intake, Anand and Dua found that lateral hypothalamic stimulation increased food intake, medial hypothalamic stimulation decreased feeding and stimulation of other areas was without effect (10). These results substantiate earlier proposals for a lateral hypothalamic "feeding center" and a medial "satiety center"(9).

Similar results have been obtained in rats; electrical stimulation of lateral hypothalamus generally increases food intake (184, 205), even in sated animals (147), whereas VMN stimulation decreases feeding (147, 184, 205). Furthermore, by recording the spontaneous unit discharges from the VMN and LHA simultaneously,

a reciprocal relation between these two hypothalamic areas has been demonstrated (154).

Conclusions drawn from data obtained by electrical stimulation should be interpreted with caution. Although hungry rats can be stopped from eating with electrical stimulation of VMN, evidence suggests that this may be due to the noxious nature of the stimuli rather than to hunger per se (122). Sclafani and Maul have questioned the relationship between the VMN and lateral hypothalamus (180). They inhibited feeding in hungry rats with stimulation of VMN, but could not alter this inhibitory effect with either knife cuts between the VMN and LHA or LHA ablation. These findings suggested that the VMN does not inhibit the LHA. However, due to possible spread, the authors admitted that electrical stimulation may not be the optimal method for studying pathways involved in the control of food intake.

Role of extrahypothalamic structures - Incidental findings in both patients and animals recuperating from brain surgery, especially surgery involving either the frontal or temporal lobes, have pointed to disturbances in feeding behavior (7). Studies involving cats and monkeys demonstrated that frontal lobe lesions may result in decreased or increased food intake, depending on whether the orbital cortex was destroyed or spared, respectively (11).

Furthermore, amygdalectomy caused temporary aphagia, while complete temporal lobectomy produced mild hyperphagia (11). These observations imply that structures in the frontal and temporal lobes, including the limbic system, can influence food intake. In reviewing the literature, Anand (7), found evidence for both amygdalar facilitation and inhibition of food intake. More extensive research suggests that the corticomedial amygdala inhibits food intake (87) and that this inhibition is mediated via connections with the VMN through the stria terminalis (199). The lateral amygdala apparently augments feeding (71).

Other CNS structures also appear to affect feeding. Morgane produced aphagia in animals by interrupting pallido-hypothalamic fibers (146). Dorsolateral hippocampal electrical stimulation resulted in feeding immediately after cessation of stimulation, suggesting to Milgram that this part of the hippocampus normally inhibits eating and that post-stimulation feeding is caused by rebound excitation of the LHA (140). Gold suggested that a functional connection exists between the LHA and the mesencephalon after observing that a unilateral mesencephalic lesion in combination with a lesion in the contralateral lateral hypothalamus produced prolonged aphagia (79). Hyperphagia has also occurred from mesencephalic lesions (86). At present, the physiological roles of these myriad extrahypothalamic structures and pathways in the control of food intake are not known.

Summary - Accumulated evidence suggests that there are two opposing mechanisms located in or operating through the hypothalamus which are essential for the control of food intake. The lateral hypothalamic area, classically designated the "feeding center," contains those neural structures which initiate feeding, whereas a medial hypothalamic "satiety center" is important in restraining food intake. It appears that the medial hypothalamus manifests its effects by modulating the activity of the lateral area. The lateral hypothalamic region is presumed to exert its effects through projections to those neurons in the brainstem and spinal cord that are ultimately responsible for actual feeding behavior. Higher forebrain structures probably influence feeding behavior through these hypothalamic mechanisms. Several theories have been proposed concerning afferent signals that regulate food intake. Inherent in these proposals are hypothalamic receptors that detect alterations in circulating metabolic intermediaries. No single theory has explained the experimental results.

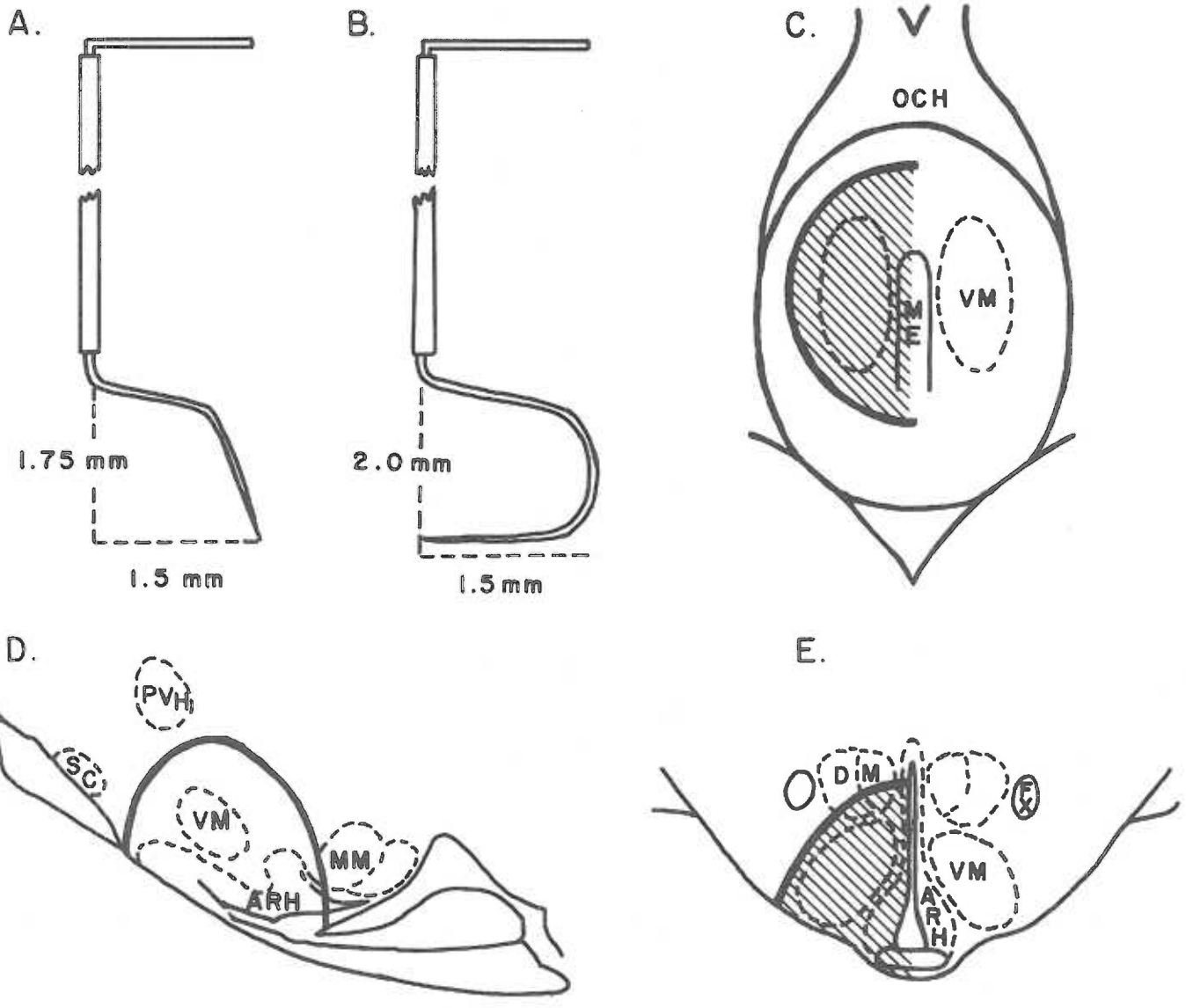
MATERIALS AND METHODS

The animals used were adult female rats (Charles River-CD), approximately 12 weeks old, which weighed 180-230 grams. The rats were allowed to acclimate for 3 weeks prior to surgery under conditions of controlled lighting (fluorescent illumination from 0400 to 1800 PST alternating with 10 hr of darkness) and temperature (26 ± 2^{o}C). Unlimited lab chow and water were available at all times.

Construction of Knives

Two different hypothalamic cuts were performed--unilateral hypothalamic ablation and unilateral hypothalamic deafferentation. Both cuts were produced by a modification of the Halász-Pupp knife (91). For the ablations, the main modification consisted of including a horizontal bar on the ventral aspect of the knife to interrupt the vascular supply to the medial basal hypothalamus. The knife was constructed from the stylet of a 20 gauge spinal tap needle. It was flattened, sharpened and bent to the dimensions shown in figure 1. A 20 gauge needle served as the holder for this knife. The deafferentations were produced by a knife constructed from 28 gauge stainless steel tubing that was flattened, sharpened and bent to the desired dimensions. Both knives were constructed with dimensions and shapes such that rotation of 180^{o} produced necrosis or disconnection

Figure 1. Schematic diagram of the neurosurgical knives used for (A) unilateral deafferentations and (B) unilateral ablations. C: Placements and dimensions of cuts as seen on the ventral surface of the brain. D: Parasagittal plane through the MBH to show approximate placement of cuts. E: Coronal plane through the MBH to show approximate boundaries of cuts.


A.
1.75 mm
1.5 mm
B.
2.0 mm
1.5 mm
C.
V
OCH
ME
VM
D.
PVH
SC
VM
MM
ARH
E.
DM
FX
VM
ARH

of the isolated hypothalamic tissue that extended in an anteroposterior direction from the posterior border of the optic chiasm to the premammillary region and laterally to the plane of the fornix (figure 1).

Surgery

The techniques for both types of hypothalamic cuts were similar. The knives were placed in the brain stereotaxically and positioned 10° posterior to the vertical plane so that the horizontal part of the blade (ablations) or tip (deafferentations) were in contact with bone. The head was fixed according to DeGroot's atlas (47). The rats were anesthetized with ether and the knife was lowered in the midline, 0.3 mm posterior to bregma, through a burr hole in the skull and through the sagittal sinus to bone. With the tip of the knife facing anteriorly and being held securely against bone, it was rotated 180° to the right to produce the desired cut.

Ten animals were randomly assigned to each of four experimental groups: intact controls, sham-operated controls, unilateral medial basal hypothalamic deafferentation and unilateral medial basal hypothalamic ablation. Sham operation involved lowering a knife, with the tip facing anteriorly, in the midline to the base of the skull without rotation. With the exception of certain experimental conditions described below, the animals were housed 4 per cage under preoperative conditions of lighting and temperature. Three days

preceding each experiment in which blood was collected, animals were housed individually and the animal quarters locked 24 hr before each study to standardize conditions for non-stress hormonal levels.

Experiment 1: Effects of Unilateral MBH Knife Cuts on the Plasma LH Surge During Proestrus, Ovulation and Ovarian Compensatory Hypertrophy

Vaginal smears were taken 5 days per week for two weeks before surgery and 5 days per week thereafter to assess pituitary-ovarian function. Animals were checked 6 weeks postoperatively for two successive normal cycles. To measure plasma LH levels existing prior to the ovulatory surge, a 0.5 ml sample of blood was collected from the tail vein at 10 am on the morning of proestrus from those rats that cycled normally. Hourly 0.5 ml blood samples were similarly obtained from the tail vein at 1400, 1500, 1600, 1700, 1800 and 2000 to assess the magnitude and duration of the LH surge. Tail vein bleeding involved preparing the animals 3 days before sampling by making a 5 mm incision in the dorsal vein of the tail. This incision was reopened at the time of bleeding and blood was drawn by suction into an EDTA-rinsed Pasteur pipette within 3 min following cage opening and without the use of anesthesia. Because more than 3 min are required for stress to change plasma LH levels (124), these samples were used to assess non-stress LH concentrations. The blood samples were chilled and centrifuged. The plasma was collected,

rapidly frozen and stored at -15°C for RIA of LH as described below. On the day of vaginal estrus that followed blood sampling, the right ovary was removed and weighed. The oviduct was dissected, the ampulla was inspected for dilation and the number of tubal ova counted to verify ovulation. At 200 days after surgery, the left ovary was removed and weighed, and the difference in weight between this and the previously removed ovary was used to assess compensatory hypertrophy.

Experiment 2: Effects of Unilateral MBH Knife Cuts on Plasma Non-Stress and Stress LH Levels

At 42 days after complete ovariectomy, the animals were bled to assess non-stress and immobilization-induced changes in LH levels. These studies were performed with a double-bleeding procedure similar to that used to assess resting and stress-induced changes in pituitary-adrenal function (208). Rats were taken individually to an adjacent room and bound firmly in the supine position on a restraining board. The time of cage opening was designated as time zero. Within 3 min of cage opening, under local procaine anesthesia, the jugular vein was exposed and a 0.5 ml blood sample was obtained. As described previously, blood collected within 3 min of initiation of stress was considered a non-stress sample. After 10 min in a recovery cage, the individual animals were reanesthetized

with ether and a final blood sample was taken at 15 min from initial cage opening. This sample was designated the stress sample. The difference between the non-stress and stress sample was used as an index of the response to 3 min immobilization stress.

Experiment 3: Effects of Unilateral MBH Knife Cuts on the 24 hr Pattern of Plasma GH Levels

The effects of hypothalamic surgery on non-stress GH levels were assessed by sequential blood sampling for 24 hr at 125 days after surgery. Tail vein bleeding was used for collecting samples as described for Experiment 1. Samples were taken from all animals every 6 hr, at 0800, 1400, 2000 and 0200. Each blood sample was chilled and centrifuged and plasma removed and rapidly frozen for eventual RIA of GH as described below.

Experiment 4: Effects of Unilateral MBH Knife Cuts on Plasma Non-Stress and Stress GH Levels

Non-stress and stress plasma GH levels were determined by the double-bleeding procedure described above. Both 3 min immobilization and 3 min ether were used as stressors. The first method was described above under LH experiments and was performed on ovariectomized animals 275 days postoperatively. The latter procedure was performed at 100 days after surgery and involved transferring individual animals from separate cages to an adjacent

room where they were placed in a saturated ether environment. After anesthesia was achieved, the jugular vein was exposed and a 0.5 ml blood sample was removed in less than 3 min from time of cage opening and used to determine non-stress GH levels (141). The animals were then placed in a recovery cage for 10 min, and the stress sample was obtained at 15 min after reanesthetization with ether. Each sample was processed in the previously described manner for RIA of GH and differences in GH levels between non-stress and stress samples were used as an index of the response to 3 min ether stress.

Experiment 5: Effects of Unilateral MBH Knife Cuts on the Circadian Rhythm of Pituitary-Adrenal Function

The pattern of changes in plasma corticosterone (Cpd. B) levels was investigated by serially sampling tail vein blood for a 24 hr period at 125 days postoperatively. The animals were prepared and bled by the method described in Experiment 1. A 6 hr interval was deemed adequate to assess the circadian rhythm of plasma Cpd. B levels. Bleeding times were 0800, 1400, 2000 and 0200. Samples were drawn within less than 3 min of cage opening to obtain non-stress levels. Earlier studies have shown that Cpd. B concentration in plasma obtained within 3 min following onset of stress is a valid index of non-stress pituitary-adrenal function (208). All blood was

centrifuged and the separated plasma was used to measure corticosterone levels fluorometrically as described below. Cpd. B levels were used as an index of ACTH secretion.

Experiment 6: Effects of Unilateral MBH Knife Cuts on Plasma Non-Stress and Stress Corticosterone Levels

These studies of pituitary-adrenal function were performed concurrently with those involving LH and GH. The effects of unilateral lesions on stress levels of corticosterone was investigated by the 3 min immobilization stress and the 3 min ether stress using the double bleeding procedures described above in Experiments 2 and 4, respectively. Differences in corticosterone levels in the non-stress and stress blood samples were used as indices of pituitary-adrenal responses to stress.

Assessment of Growth, Food Intake, and Obesity

Body weights and incisor-anal lengths, used as an index of body length, were taken at each of the two weeks before surgery, at the time of surgery and at regular intervals thereafter; body weights were measured bimonthly and body lengths determined every 3 weeks. Incisor-anal lengths were obtained by anesthetizing the animals with ether, inserting the incisors on a hroizontal bar, which corresponded to the "0" point of a superimposed metric ruler, and measuring each

animal in the supine position from the incisors to the anus. Lee's Nutrition Index (127) was calculated for each animal to assess obesity. Starting 1 week post-operatively, food intake of each animal was measured approximately every 40 days. Rats were put in individual cages and each given a measured aliquot of food. The food remaining at the end of 24 hr was weighed and the difference between this weight and the initial food weight was used to estimate 24 hr food intake.

Assays

The ovine:ovine method (152) was used for RIA of LH. Sheep LH for iodination (LER-1213-a) was supplied by Dr. Leo Reichert and the ovine antibody obtained from Dr. G. Niswender. NIAMDD-Rat LH-RP-1, with a biologic potency equivalent to 0.03 NIH-LH-S1, was used as a standard. The LH antigen:antibody complexes were precipitated with goat-anti-rabbit gamma globulin. The precipitates were counted in a Nuclear-Chicago Auto Gamma well-type scintillation counter. A computer program (Packard instruments) was used to calculate the standard curve. Samples were run at 2 dilutions in a single assay and results were averaged. The sensitivity of the assay was such that 25-50 ng/ml could be detected. Inter- and intra-assay coefficients of variability were 6% and 5%, respectively.

Plasma GH was measured by a modification of the double-antibody RIA described by Schalch and Reichlin (177), and Birge et al.

(22). Samples were run at 2 dilutions and the values averaged. The rat GH for iodination (Rat GH-I-2), rat GH reference preparation (Rat GH-RP-1 with a biologic potency equivalent to 0.6 IU bovine GH/mg), and anti-rat GH serum (monkey) (A-Rat GH-S-2) used in these experiments were obtained from NIAMDD, Rat Pituitary Hormone Program. The labeled GH antigen:antibody complexes were precipitated with goat-anti-monkey gamma globulin and the precipitates counted in a Nuclear-Chicago Auto Gamma well-type scintillation counter. A computer program was used to calculate the standard curve (Packard instruments). Inter- and intra-assay coefficients of variability were 13% and 4.7%, respectively.

Plasma levels of corticosterone were measured with a micro-fluorometric method (77). Correction for residual fluorescence was not made. However, in this laboratory, fluorescence measured in plasma from adrenalectomized female rats is equivalent to approximately 6 μg/100ml plasma.

Autopsy

At the conclusion of all studies, approximately 300 days after surgery, the rats were killed by decapitation. Hearts, kidneys, adrenals and pituitaries were removed and weighed. Brains were removed and placed in a 10% formalin solution for fixation. Each brain was processed for histological examination; alternate 50 μ

frozen sections were stained with toluidine blue and used for evaluating lesion morphology.

Statistics

In each study described above, treatments were assigned and performed and data evaluated by a completely randomized design. Differences between groups were evaluated by two-way analysis of variance for repeated measures (202) and the multiple range test of Newman-Keuls (151). Heterogeneity of values among groups was treated by log transformation of the data.

RESULTS

Brain Histology

Histological examination of brain sections necessitated revision of the originally-designated experimental groups. Whereas, ten rats were originally assigned to the unilateral deafferentation group, only two had hypothalamic cuts that satisfied the previously described criteria. Data from this group are not included in this presentation. Five rats with presumed unilateral deafferentations or ablations had incomplete ablations and are therefore designated as a separate group for purposes of data presentation and analysis. A cut was considered incomplete if it did not eliminate all of the tissue indicated in figure 1. Some of these incomplete cuts did not conform to the anteroposterior boundaries, whereas others did not extend far enough laterally. Eleven rats had acceptable unilateral ablations. However, there was some heterogeneity even in this group. Seven rats had what appeared to be complete unilateral ablation of MBH. In these animals, the lesion extended laterally to the plane of the fornix, there was no histological evidence of VMN or arcuate nuclei and the median eminence appeared intact. Four rats had lesions that were similar, but scattered, small clusters of neurons of the arcuate nuclear complex were present in some sections. These residual arcuate neurons appeared to be isolated from surrounding brain tissue. Because

the results in animals with persisting nests of arcuate neurons did not differ from those with complete elimination of this nucleus, the data from these two groups were pooled. All of the sections examined demonstrated unilateral surgical scars which did not extend to the opposite side of the brain. The lesions reached the base of the brain in most sections of most brains. However, in a few sections in some brains, there was a residual 0.1-0.3 mm shell of tissue on the ventral surface. This tissue did not appear to affect the endpoints studied. Figure 2 shows photomicrographs of representative brain sections from an animal with unilateral ablation in which only a few neurons of the arcuate nucleus remained. Figure 3 shows photomicrographs of representative brain sections from an animal with unilateral ablation in which no histological evidence of the arcuate-VMN complex remained.

Experiment 1: Effects of Unilateral MBH Knife Cuts on the Plasma LH Surge During Proestrus, Ovulation and Ovarian Compensatory Hypertrophy

Serial blood samples were taken by tail vein on the day of proestrus from all animals which showed at least two normal estrous cycles. The group data are presented in figure 4. The collection of serial blood samples from a tail vein did not appear to interfere with the proestrous LH surge because both the intact and sham-operated control groups showed marked increases in LH levels during the

Figure 2. Coronal brain sections of an animal with arcuate nucleus neurons remaining on the lesioned side. Sections A, B and C represent rostral, middle and caudal parts of the lesion, respectively. Arrows point to the lateral boundary of the lesion. Abbreviations in this figure and in figure 3 are as follows: ARC, arcuate nucleus; FX, fornix; ME, median eminence; MT, mammillothalamic tract; OT, optic tract; VMN, ventromedial nucleus; V III, 3rd ventricle.

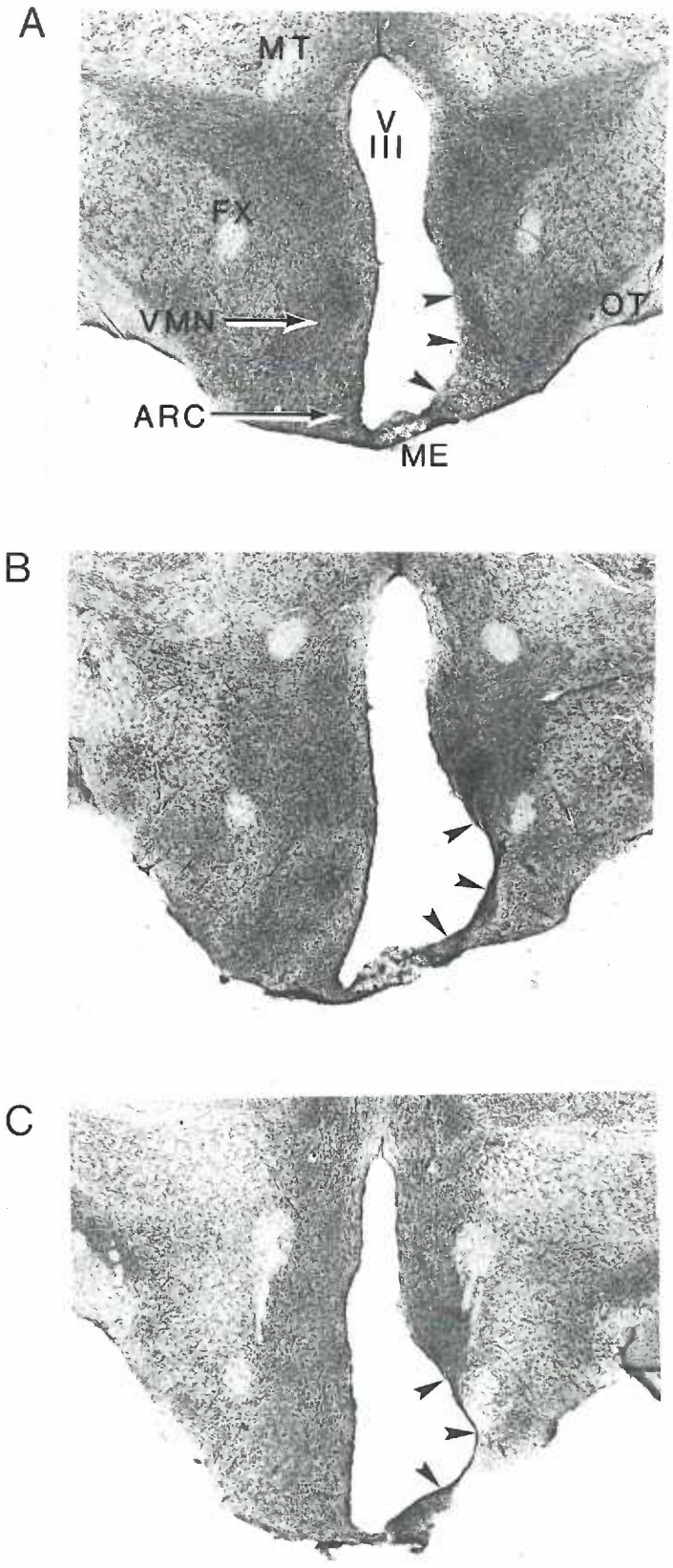

A
MT
V
III
FX
VMN
OT
ARC
ME
B
C

Figure 3. Coronal brain sections of an animal with no arcuate nucleus neurons remaining on the lesioned side. Arrows point to lesion.

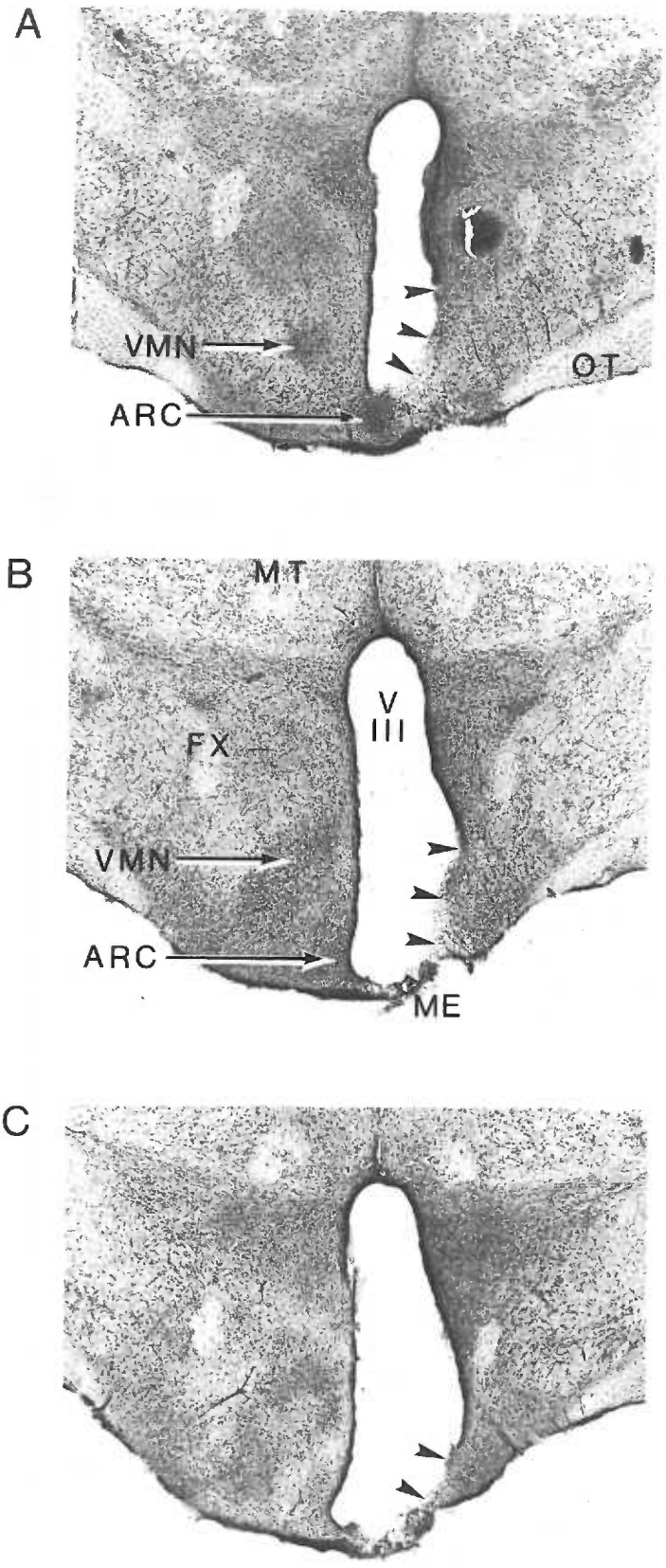
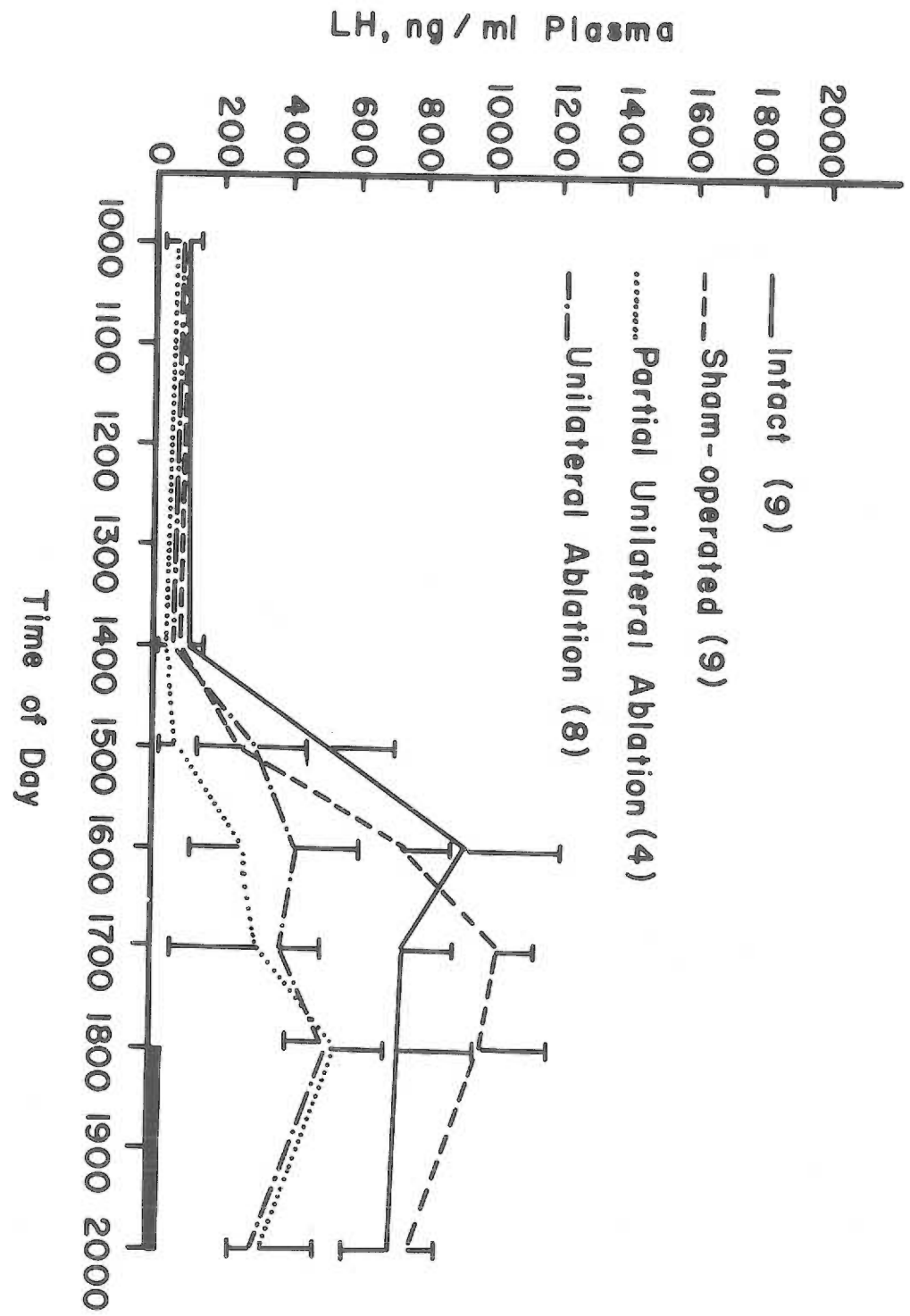

A
VMN
ARC
OT
B
MT
V
III
FX
VMN
ARC
ME
C

Figure 4. Plasma LH during the day of proestrus in intact, sham-operated, unilateral ablated and partial unilateral ablated rats. In this and subsequent figures, the black horizontal bar denotes dark period; vertical lines represent the standard errors of the means.


LH, ng/ml Plasma
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Intact (9)
Sham-operated (9)
Partial Unilateral Ablation (4)
Unilateral Ablation (8)
1000 1100 1200 1300 1400 1500 1600 1700 1800 1900 2000
Time of Day

afternoon. In fact, two-way analysis of variance indicated that all groups showed significant ($p < 0.01$) elevations in LH after 1400. Although peaks in LH levels observed in both experimental groups were approximately 50% of those in controls, there was considerable variation in the timing, duration and amplitude of the changes in individual rats. Because of this variation, there were no overall effects of treatments. The LH levels of individual rats from the several treatment groups are presented in Table 1.

The ovulatory responses associated with the proestrous LH patterns described above were assessed by performing a right ovariectomy on the subsequent day of vaginal estrus and examining the oviduct for the number of tubal ova. The results of these observations are presented in Table 2. Complete ovulation occurred in all of the intact and sham-operated animals. Approximately 87% of the unilateral-ablated rats ovulated completely. Only 25% of the animals in the partial ablation group showed complete ovulation. The animals that did not ovulate in these groups had previously shown long periods of diestrus with occasional normal cycles and two of these animals were in diestrus during the experiment.

Approximately 200 days after right ovariectomy, the left ovary was removed and weighed to assess ovarian compensatory hypertrophy. As shown in Table 3, all groups showed evidence of such hypertrophy. The relative weights of the first ovaries removed from

Table 1. Effects of unilateral MBH ablation on the LH surge during proestrus.

Group	Animal No.	Time of Sampling 1000	1400	1500	1600	1700	1800	2000
Intact								
	6	--	73.8[a]	324.2	1826.1	1170.4	1341.1	1084.4
	11	49.7	107.0	99.3	554.1	985.1	889.8	627.0
	15	26.2	137.6	981.0	705.5	895.3	1163.9	572.7
	20	14.1	131.3	1634.0	1357.5	1255.7	2033.2	1183.2
	22	120.2	118.0	1038.0	2319.4	1178.1	722.4	798.3
	25	228.6	176.0	83.2	162.3	484.2	102.5	877.9
	29	75.7	117.7	150.2	494.9	137.0	386.2	109.2
	36	18.4	94.3	44.2	192.9	156.8	26.7	20.0
	39	134.5	--	34.9	46.3	507.7	44.1	1161.5
	mean ± SEM	83.4 ± 26.2	106.4 ± 16.4	487.7 ± 194.3	937.1 ± 287.3	752.3 ± 146.7	746.1 ± 228.9	714.9 ± 142.7
Sham-operated								
	3	66.1	179.5	146.4	878.0	1553.9	1460.1	1093.4
	12	184.4	80.0	930.9	1461.1	1253.9	1134.1	756.3
	16	37.2	61.4	104.1	213.2	548.5	676.5	869.1
	19	32.2	50.3	16.1	50.8	60.6	514.4	555.5
	24	191.2	47.4	141.3	--	246.1	291.6	478.0
	28	85.0	76.4	686.6	957.5	1225.0	1179.5	732.0
	31	--	39.3	152.1	1638.1	2900.3	1989.3	1292.5
	34	45.3	116.6	78.8	360.1	546.9	362.2	557.7
	37	118.5	99.1	123.3	369.7	919.7	1281.0	631.1
	mean ± SEM	95.0 ± 22.6	83.3 ± 14.7	264.4 ± 105.8	741.1 ± 208.2	1028.3 ± 286.1	987.6 ± 188.8	774.0 ± 90.1

Table 1. (Continued) Effects of unilateral MBH ablation on the LH surge during proestrus.

Group	Animal No.	Time of Sampling 1000	1400	1500	1600	1700	1800	2000
Partial Unilateral Ablation	9[b]	156.5[a]	29.7	61.0	120.9	56.1	256.6	122.9
	18[b]	83.9	33.3	150.3	761.9	1098.2	1435.4	718.7
	38[b]	25.9	68.4	75.6	240.5	111.5	237.6	187.5
	45[b]	14.2	53.6	73.6	36.3	68.9	220.7	222.8
	mean ± SEM	70.1 ± 32.6	46.3 ± 9.1	90.1 ± 20.3	289.9 ± 162.8	333.7 ± 255.1	537.6 ± 299.4	313.0 ± 136.8
Unilateral Ablation	1	36.2	118.7	193.6	332.4	398.5	219.3	346.0
	13	144.2	108.1	1430.5	1645.0	1066.6	1062.2	598.7
	14	58.8	85.4	287.5	293.0	236.2	371.8	196.2
	21	119.4	85.2	232.2	553.2	842.2	900.2	389.9
	30[b]	197.6	153.1	126.4	274.5	88.4	123.3	264.3
	40	141.8	107.0	76.3	204.0	131.6	264.9	88.8
	41	31.1	2.6	89.4	138.7	117.2	1134.8	129.2
	46	51.9	33.0	39.0	150.2	252.5	181.9	542.3
	mean ± SEM	97.1 ± 21.7	86.6 ± 17.0	309.4 ± 162.9	447.6 ± 177.2	391.6 ± 129.3	532.3 ± 150.3	319.4 ± 65.6

[a] LH concentration: ng/ml plasma

[b] rats with blocked ovulation

Table 2. Effects of unilateral MBH ablation on incidence of ovulation.

Group	No.	Complete[a] Ovulation		Partial[b] Ovulation		Ovulation Blocked	
		No.	%	No.	%	No.	%
Intact	9	9	100	0	0	0	0
Sham-operated	9	9	100	0	0	0	0
Unilateral Ablation	8	7	87.5	0	0	1	12.5
Partial Unilateral Ablation	4	1	25	0	0	3	75

[a] 3-8 ova per Fallopian tube

[b] < 3 ova per Fallopian tube

Table 3. Effects of unilateral MBH ablation on ovarian compensatory hypertrophy.

Group	No.	Ovarian weights, mg/100 gm body weight		
		1st ovary	2nd ovary	% ovarian hypertrophy
Intact	9	10.7 ± 0.8[a]	13.9 ± 0.9[d]	36.0 ± 17.1
Sham-operated	9	11.9 ± 1.4	13.4 ± 1.5[c]	21.2 ± 14.1
Unilateral Ablation	8	8.0 ± 0.8[b]	11.1 ± 1.7[d]	35.1 ± 12.9
Partial Unilateral Ablation	4	9.7 ± 1.7	12.1 ± 1.2[c]	52.4 ± 47.8

[a] mean ± SEM

[b] $p < 0.05$ vs sham group

[c] 2nd ovary vs 1st ovary $p < 0.05$

[d] 2nd ovary vs 1st ovary $p < 0.01$

the unilateral-ablated group were lower ($p < 0.05$) than those from sham-operated controls. Otherwise, ovarian weights were comparable in the several groups.

Experiment 2: Effects of Unilateral MBH Knife Cuts on Plasma Non-Stress and Stress LH Levels

The effect of 3 min immobilization stress on plasma LH concentrations is illustrated in figure 5. This experiment was performed at 42 days after complete ovariectomy. The mean non-stress plasma level of LH in rats with unilateral ablations was less than 50% of those in the other groups ($p < 0.01$). At 15 min after stress, the mean LH concentration in unilateral-ablated rats was also significantly lower than in the other groups ($p < 0.05$). Using the paired-t test, all groups showed significant stress-evoked decreases in LH concentrations. Comparison of the stress-induced LH decrements using two-way analysis of variance demonstrated no differences between the four groups of animals.

Experiment 3: Effects of Unilateral MBH Knife Cuts on the 24 Hr Pattern of Plasma GH Levels

Sequential blood samples were taken by tail vein over a 24 hr period from all animals to assess the temporal patterns and levels of plasma GH concentrations. The effect of unilateral MBH knife cuts on 24 hr GH levels is illustrated in figure 6. There was considerable

Figure 5. Effect of 3 min immobilization stress on plasma LH levels in ovariectomized intact, sham-operated, unilateral ablated and partial unilateral ablated rats.

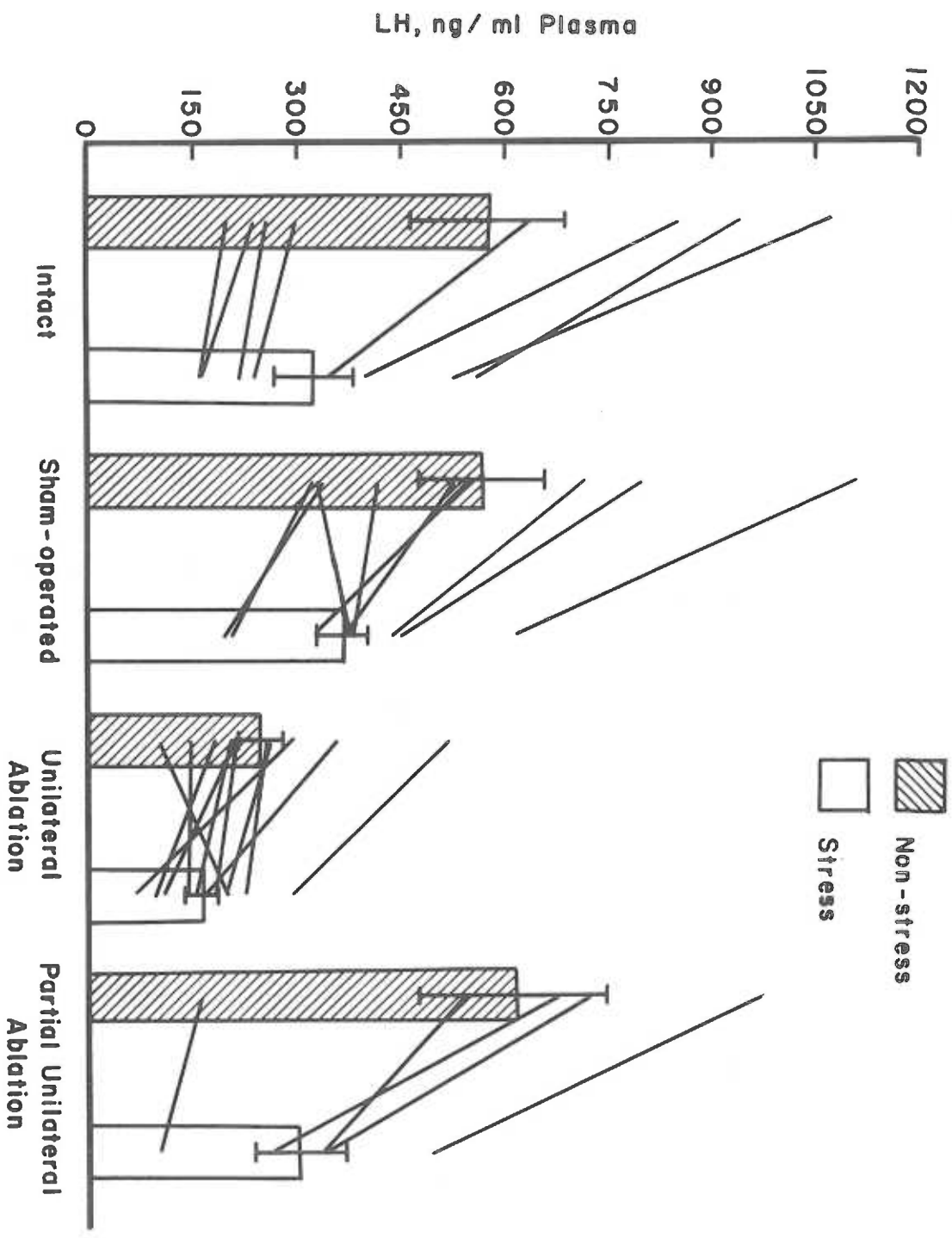
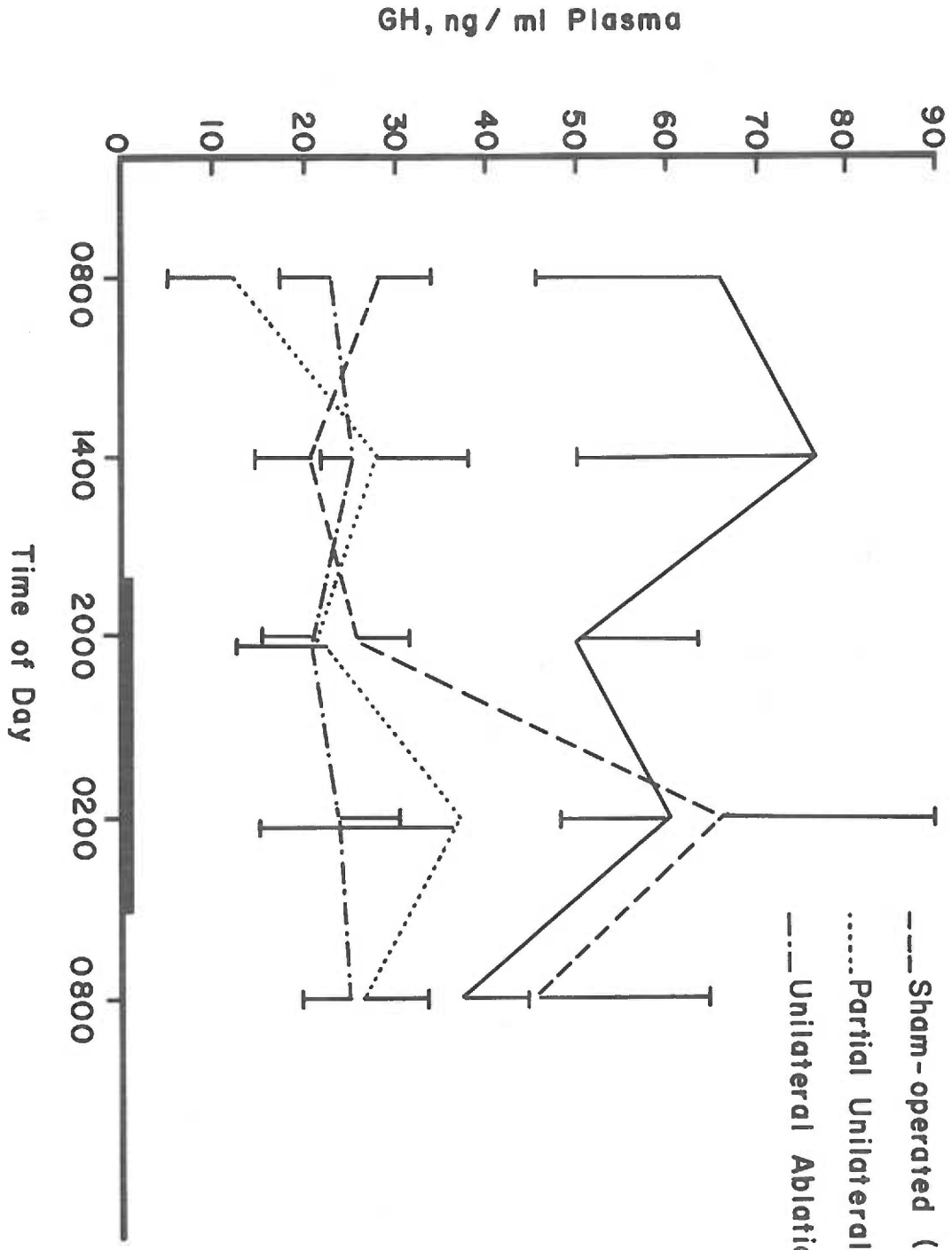

LH, ng/ ml Plasma
0
150
300
450
600
750
900
1050
1200
Intact
Sham-operated
Unilateral Ablation
Partial Unilateral Ablation
Non-stress
Stress

Figure 6. Non-stress levels of GH at different times during the 24 hr light-dark cycle in intact, sham-operated, unilateral ablated and partial unilateral ablated rats.

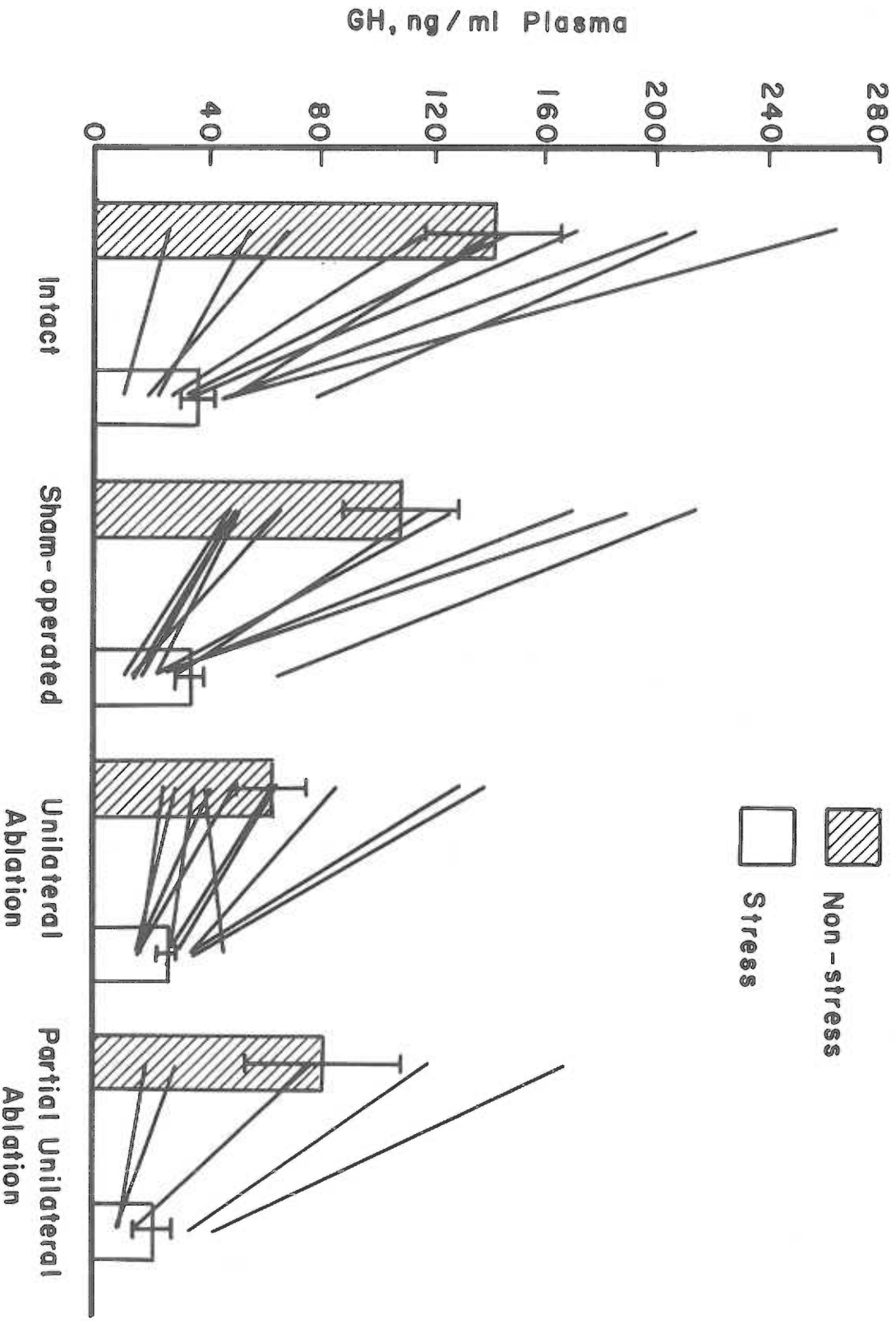

GH, ng / ml Plasma
0
10
20
30
40
50
60
70
80
90
0800
1400
2000
0200
0800
Time of Day
Intact (10)
Sham-operated (9)
Partial Unilateral Ablation (5)
Unilateral Ablation (11)

variation in non-stress GH concentrations in animals of all groups at all sampling times. There were no statistically significant, variations with time in any of the groups. Therefore, there was no evidence for diurnal variation. Two-way analysis of variance demonstrated that the GH levels in the unilaterally- and partial unilaterally-ablated animals were lower ($p < 0.05$) than those of the intact controls. The levels in these experimental groups were approximately 50% of those in the intact controls. GH concentrations in the sham-operated rats did not differ from those of the other groups.

Experiment 4: Effects of Unilateral MBH Knife Cuts on Plasma Non-Stress and Stress GH Levels

The effect of unilateral MBH knife cuts on the GH response to stress was studied using two different stressors. The response to 3 min ether inhalation is demonstrated in figure 7. The only significant difference ($p < 0.05$) in non-stress GH levels was between the unilateral ablated rats and intact controls. All groups showed significant decreases in plasma GH concentrations 15 min after stress, and there were no group differences in stress GH levels. With the exception of one rat in the unilateral ablation group which showed a modest increase, all animals demonstrated decreases in GH levels in response to stress.

Figure 7. Effect of 3 min ether stress on plasma GH levels in intact, sham-operated, unilateral ablated and partial unilateral ablated rats.


GH, ng/ml Plasma
0
40
80
120
160
200
240
280
Intact
Sham-operated
Unilateral Ablation
Partial Unilateral Ablation
Non-stress
Stress

The effects of unilateral MBH knife cuts on the GH response to 3 min immobilization stress are presented in figure 8. For unknown reasons, both non-stress and stress levels of GH were much lower in this experiment than in the preceding study. Non-stress GH concentrations in intact controls were approximately 4 times higher ($p < 0.01$) than in partially-ablated animals and about 2 times higher ($p < 0.05$) than in sham-operated and unilateral-ablated rats. GH levels 15 min after stress were similar in all groups. The stress-evoked GH response to immobilization was significant in all groups ($p < 0.05$ for partially-ablated rats; $p < 0.01$ for other groups).

Experiment 5: Effects of Unilateral MBH Knife Cuts on the Circadian Rhythm of Pituitary-Adrenal Function

The effects of unilateral MBH knife cuts on the 24 hr pattern of non-stress plasma corticosterone levels are summarized in figure 9. The highest corticosterone levels were observed at 1400 and 2000 in all groups. The true zenith was possibly missed because of no samples taken at 1800, the time of its occurrence. The lowest levels were detected in samples collected at 0800 and 0200. The difference between highest and lowest values was significant ($p < 0.01$) in each group. Two-way analysis of variance demonstrated significant variations with time and no differences between groups. Therefore, the data suggest that the cuts did not interfere with normal diurnal

Figure 8. Effect of 3 min immobilization stress on plasma GH levels in ovariectomized intact, sham-operated, unilateral ablated and partial unilateral ablated rats.

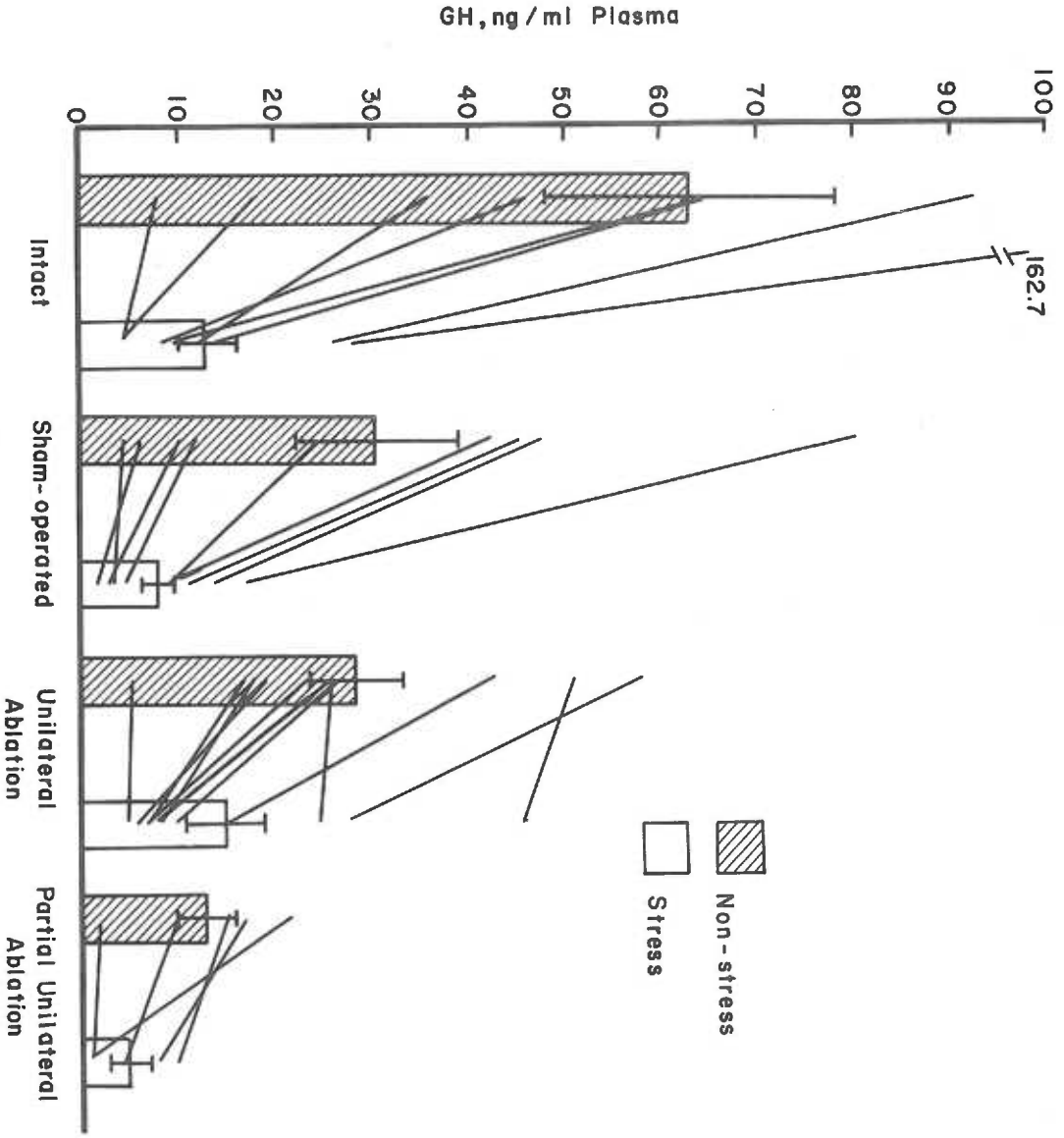
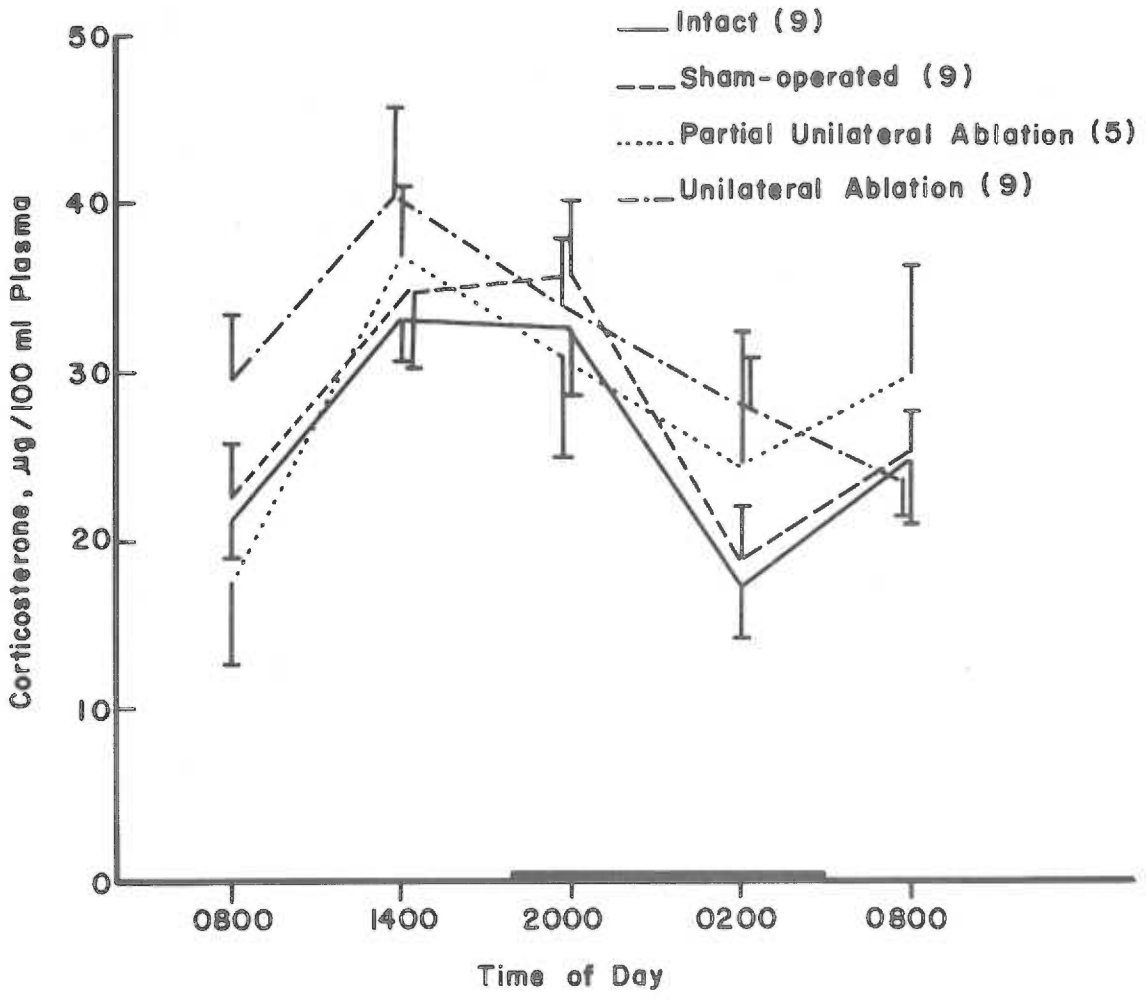

GH, ng / ml Plasma
0
10
20
30
40
50
60
70
80
90
100
162.7
Intact
Sham-operated
Unilateral Ablation
Partial Unilateral Ablation
Non-stress
Stress

Figure 9. Non-stress levels of corticosterone at different times during the 24 hr light-dark cycle in intact, sham-operated, unilateral ablated and partial unilateral ablated rats.


Intact (9)
Sham-operated (9)
Partial Unilateral Ablation (5)
Unilateral Ablation (9)
Corticosterone, µg/100 ml Plasma
50
40
30
20
10
0
0800
1400
2000
0200
0800
Time of Day

variations in pituitary-adrenal function.

Experiment 6: Effects of Unilateral MBH Knife Cuts on Plasma Non-Stress and Stress Corticosterone Levels

The effects of unilateral MBH knife cuts on responses to ether and immobilization stress are summarized in figures 10 and 11, respectively. In both experiments, these cuts failed to significantly affect non-stress or stress levels of plasma corticosterone. The increments produced in response to these two stressors were comparable in all groups.

Assessment of Growth, Food Intake and Obesity

The effects of unilateral MBH knife cuts on incisor-anal lengths are illustrated in figure 12. Body lengths and rates of growth were comparable in the intact, sham-operated and unilateral-ablated groups. Only the partially-ablated group showed stunting in response to surgery. These rats were significantly shorter than the other groups ($p < 0.05$ vs. sham-operated; $p < 0.01$ vs. other groups).

The food-consumption data are presented in figure 13. Although the unilateral-ablated group tended to eat more than controls during this study, there were no significant effects attributable to treatments.

The effects of unilateral MBH knife cuts on body weight are summarized in figure 14. During the first 21 postoperative days, all

Figure 10. Effect of 3 min ether stress on plasma corticosterone levels in intact, sham-operated, unilateral ablated and partial unilateral ablated rats.

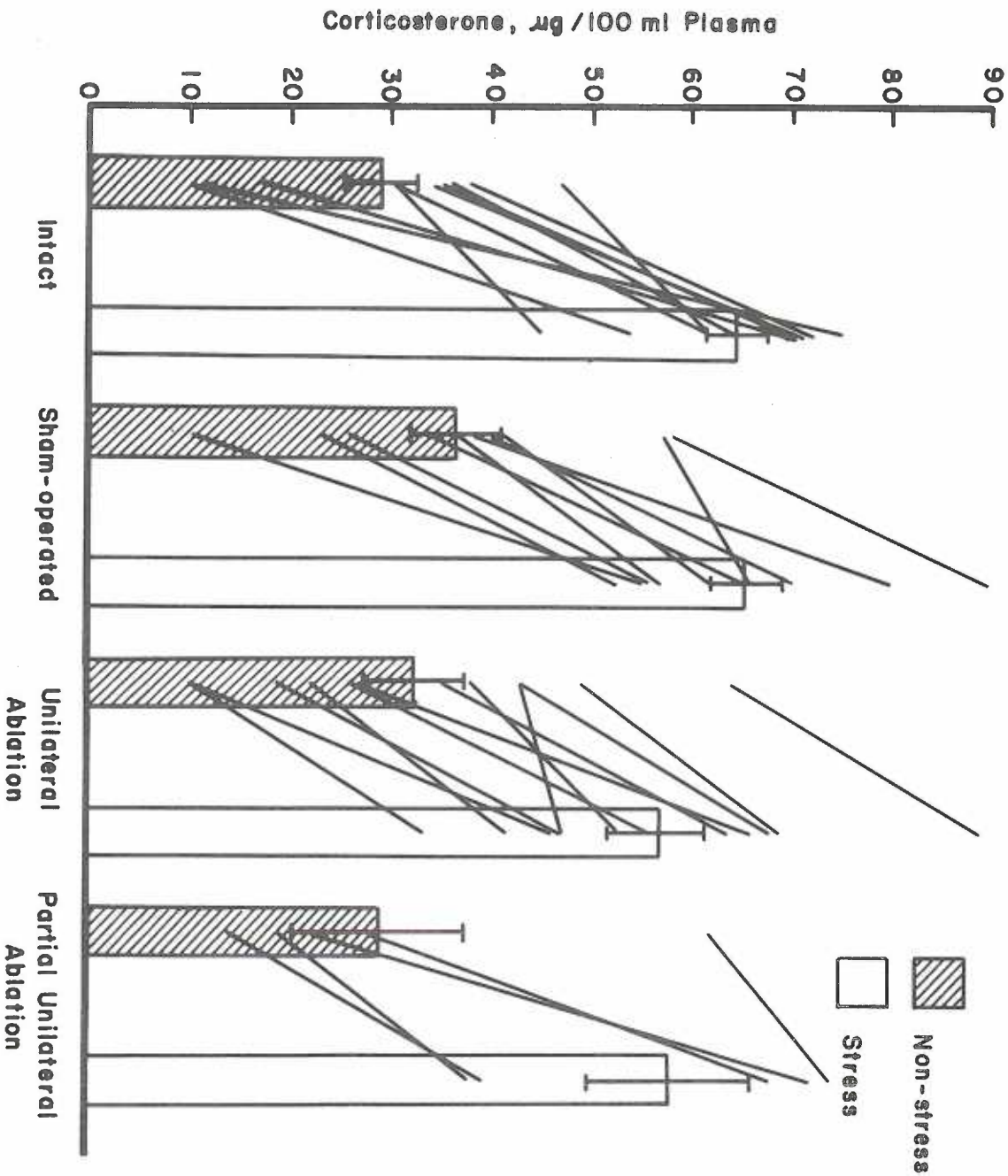

Corticosterone, µg/100 ml Plasma
0
10
20
30
40
50
60
70
80
90
Intact
Sham-operated
Unilateral Ablation
Partial Unilateral Ablation
Non-stress
Stress

Figure 11. Effect of 3 min immobilization stress on plasma corticosterone levels in ovariectomized intact, sham-operated, unilateral ablated and partial unilateral ablated rats.

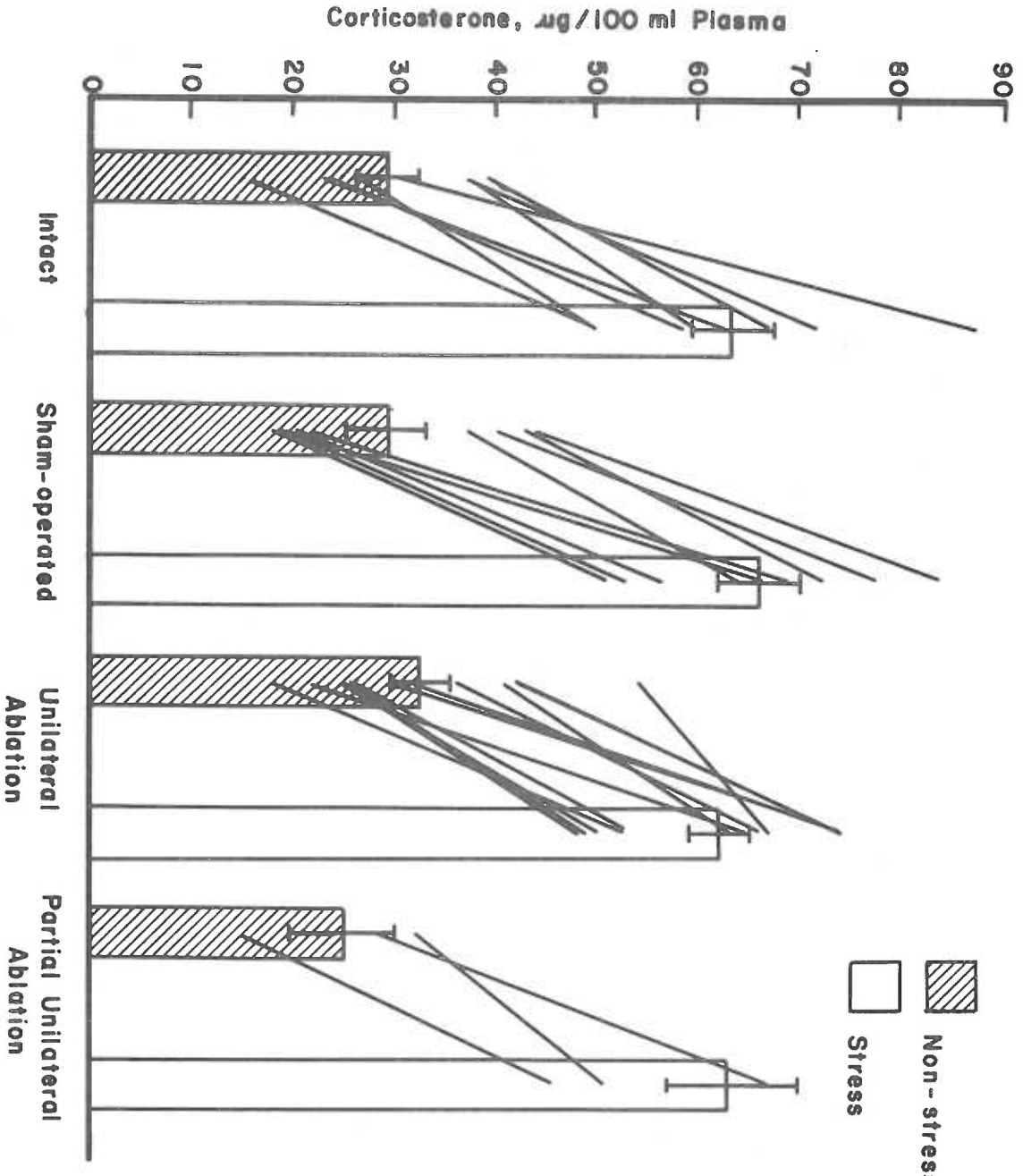

Corticosterone, µg/100 ml Plasma
0
10
20
30
40
50
60
70
80
90
Intact
Sham-operated
Unilateral Ablation
Partial Unilateral Ablation
Non-stress
Stress

Figure 12. Effects of unilateral ablation on incisor-anal lengths.

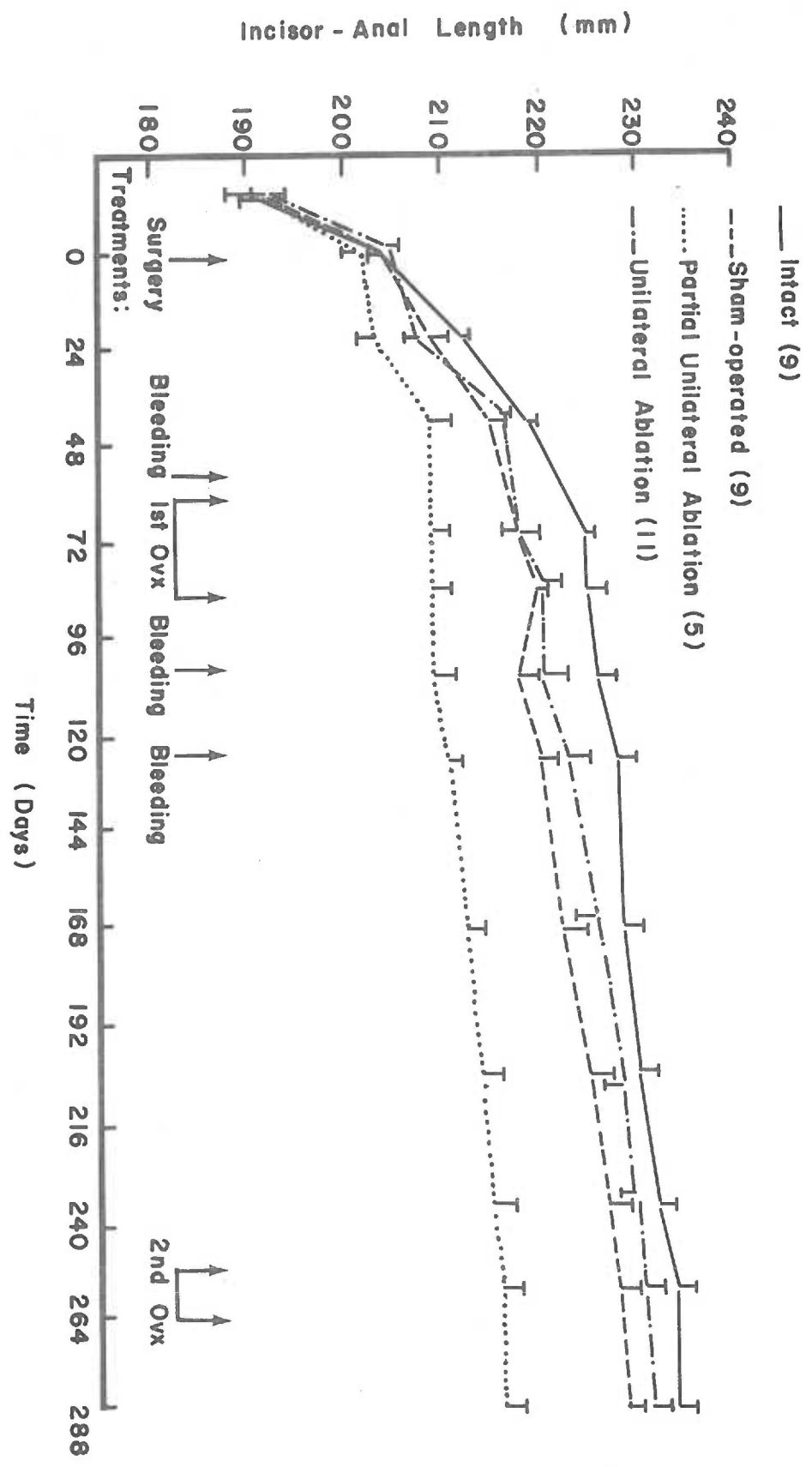

Incisor - Anal Length (mm)
240
230
220
210
200
190
180
Intact (9)
Sham-operated (9)
Partial Unilateral Ablation (5)
Unilateral Ablation (11)
Treatments:
Surgery
Bleeding
1st Ovx
Bleeding
Bleeding
2nd Ovx
0
24
48
72
96
120
144
168
192
216
240
264
288
Time (Days)

Figure 13. Effects of unilateral ablation on food intake.

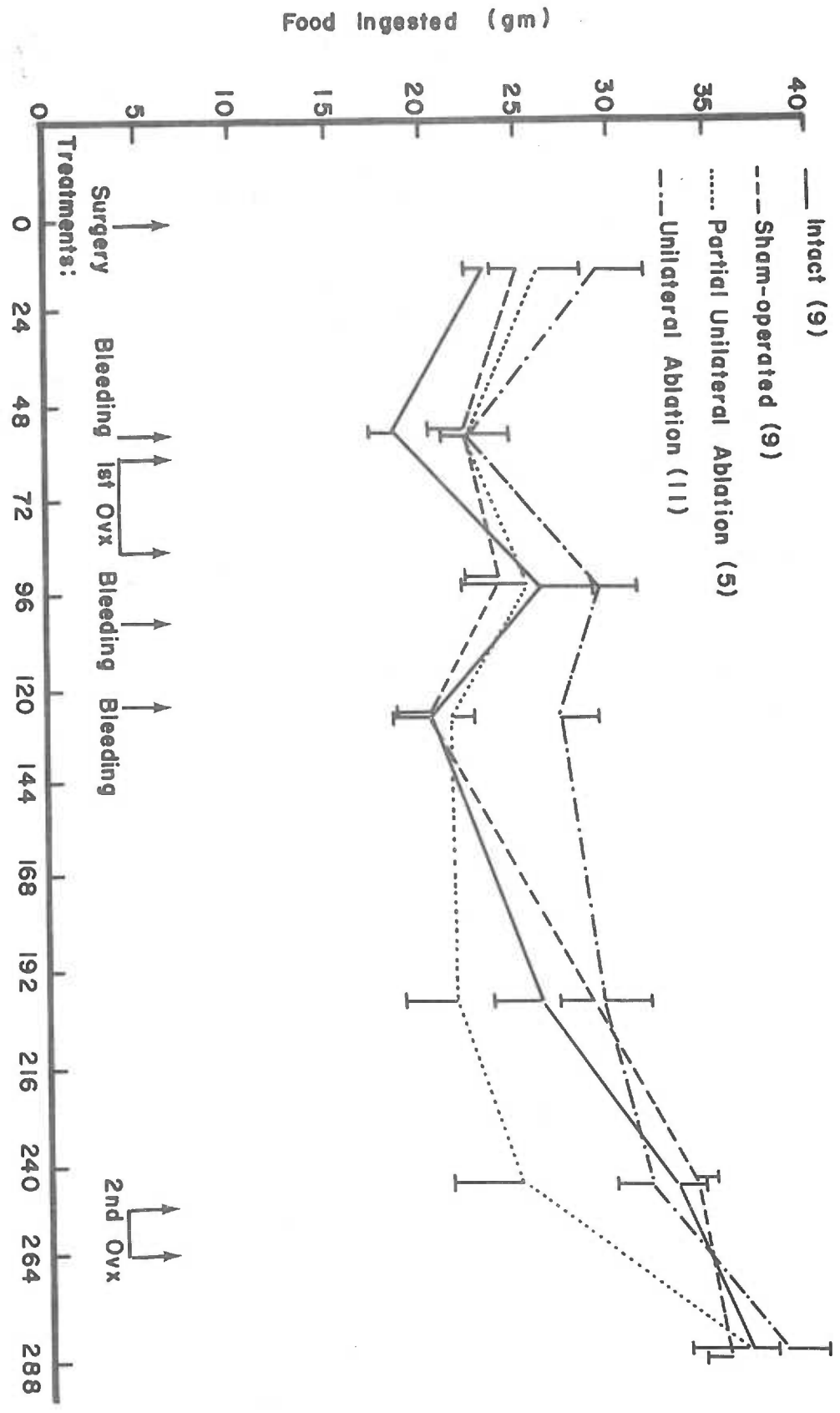
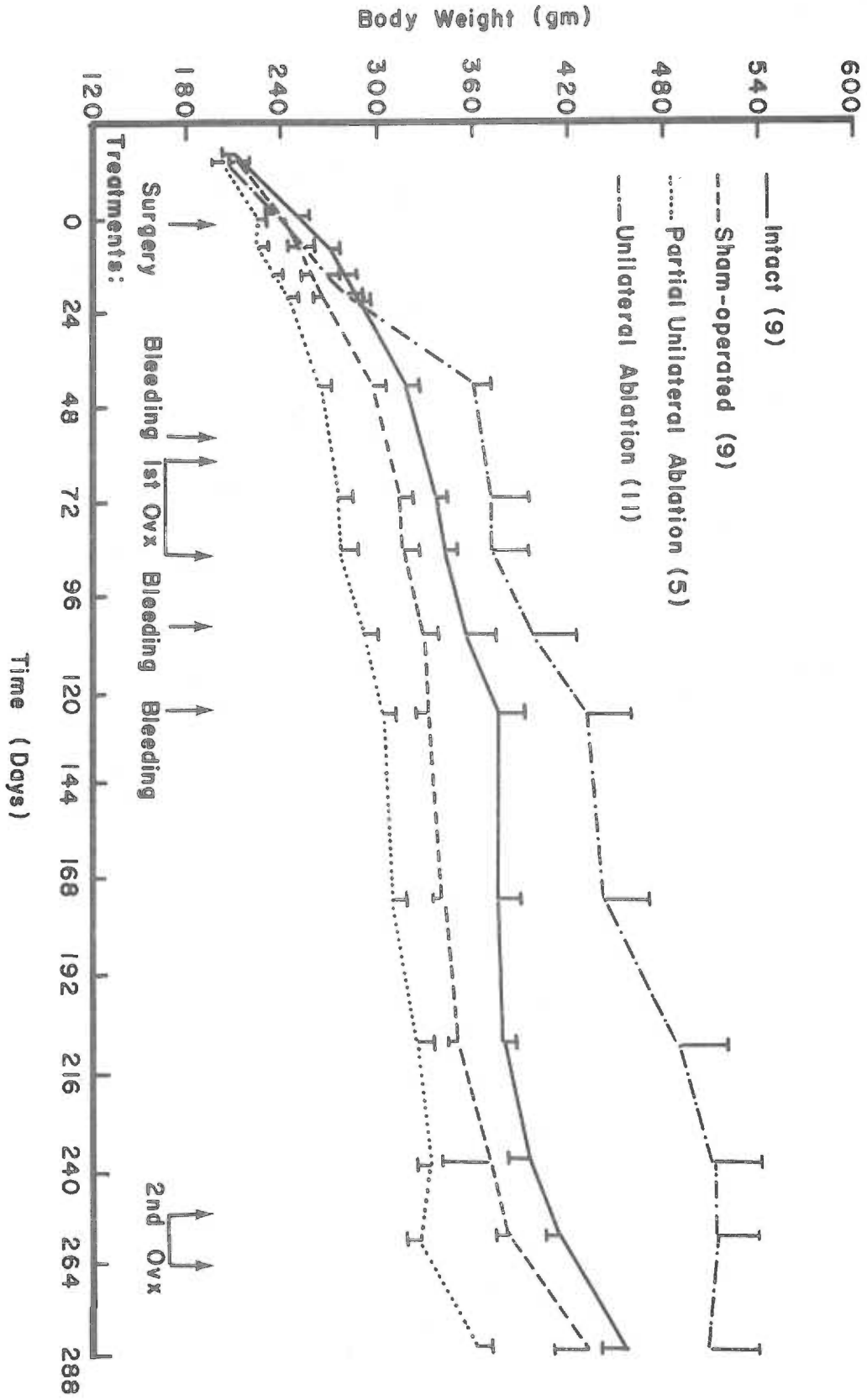

Food Ingested (gm)
0
5
10
15
20
25
30
35
40
Intact (9)
Sham-operated (9)
Partial Unilateral Ablation (5)
Unilateral Ablation (11)
Treatments:
Surgery
Bleeding
1st Ovx
Bleeding
Bleeding
2nd Ovx
0
24
48
72
96
120
144
168
192
216
240
264
288
Time (Days)

Figure 14. Effects of unilateral ablation on body weight.


Body Weight (gm)
600
540
480
420
360
300
240
180
120
Intact (9)
Sham-operated (9)
Partial Unilateral Ablation (5)
Unilateral Ablation (11)
Treatments:
Surgery
Bleeding
1st Ovx
Bleeding
Bleeding
2nd Ovx
0
24
48
72
96
120
144
168
192
216
240
264
288
Time (Days)

groups with the exception of the partially-ablated rats, increased body weights at comparable rates. The latter group gained less weight during the course of these studies than did the other groups, with the exception of the sham-operated group ($p < 0.05$ vs. intact controls; $p < 0.01$ vs. unilateral-ablated group). Two-way analysis of variance demonstrated that the unilateral-ablated animals gained more weight during these experiments than did the other groups ($p < 0.05$ vs. intacts; $p < 0.01$ vs. other groups).

Lee's Nutrition Index was computed for each animal two weeks before surgery and throughout the period of observation. The data are presented in figure 15. There were no group differences at 21 days postoperatively. Thereafter, a conspicuous divergence occurred and animals with unilateral ablations became more obese ($p < 0.01$) with time.

Autopsy

The relative weights of the anterior pituitary, heart and kidney in the unilateral ablation group were lower ($p < 0.01$) than those of both control groups. Such differences were not noted in the group with partial unilateral ablation (Table 4).

Figure 15. Effects of unilateral ablation on the obesity index:

$$\sqrt[3]{\frac{\text{body weight}}{\text{incisor-anal length}}} \times 1000$$

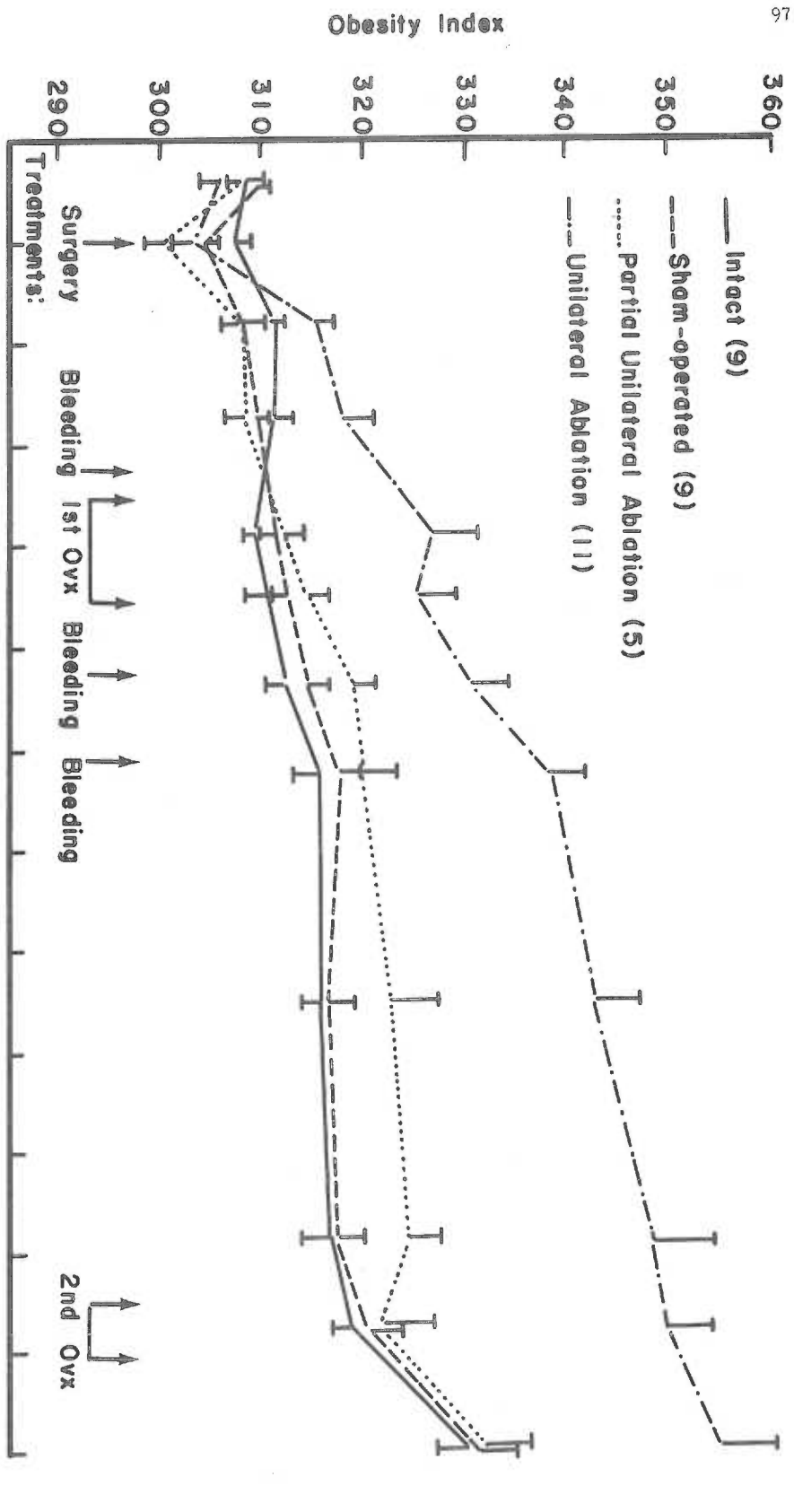

Obesity Index
360
350
340
330
320
310
300
290
Intact (9)
Sham-operated (9)
Partial Unilateral Ablation (5)
Unilateral Ablation (11)
Treatments:
Surgery
Bleeding
1st Ovx
Bleeding
Bleeding
2nd Ovx
0
24
48
72
96
120
144
168
192
216
240
264
288
Time (Days)

Table 4. Effects of unilateral MBH ablation on body and organ weights.

Group	No.	Body Weight gm	Organ weights, mg/100 gm body weight				
			Anterior Pituitary	Posterior Pituitary	Heart	Kidneys	Adrenals
Intact	9	452 ± 9[a]	3.06 ± 0.34	0.54 ± 0.09	241.2 ± 24.1	250.4 ± 4.5	14.1 ± 0.5
Sham-operated	9	432 ± 12	2.95 ± 0.14	0.54 ± 0.05	237.3 ± 9.7	239.0 ± 5.8	13.5 ± 0.6
Unilateral Ablation	11	534 ± 32[b]	1.99 ± 0.14[b]	0.39 ± 0.04	201.9 ± 6.8[b]	207.7 ± 7.1[b]	12.0 ± 1.2
Partial Unilateral Ablation	5	367 ± 7[b]	2.46 ± 0.35	0.49 ± 0.09	234.2 ± 9.2	233.2 ± 5.3	12.2 ± 1.2

[a] mean ± SEM

[b] $p<0.01$ vs intact and sham-operated controls

DISCUSSION

These experiments were designed to study the effects of both unilateral MBH isolation and ablation on several hypothalamically-mediated functions. As pointed out in Results, attempts to perform unilateral MBH isolation were successful in only 2 of 10 rats; the remaining rats in this group had lesions that produced either unilateral or partial unilateral ablations. The data from the two rats with unilateral MBH isolation were discarded. Therefore, the discussion will deal only with the 11 rats with unilateral ablation and the 5 rats with partial unilateral ablation.

The results of these investigations suggest that there is functional redundancy in the bilateral arrangement of structures that comprise the MBH in regard to several functions. It appears that ablation of one-half of the MBH leads to a 50% deficit in circulating levels of LH and GH under certain conditions. Hence, the systems that control these hormones require bilateral MBH structures for normality. Since rats with unilateral MBH ablation ovulated, maintained ovarian compensatory hypertrophy, produced LH surges on proestrus, showed normal responses to stressors and grew normally, it appears that there is a redundancy of MBH tissue for other functions. The data also suggest that a redundancy of MBH tissue exists in regard to pituitary-adrenal function. Removal of one half MBH was

compatible with maintenance of normal non-stress corticosterone levels and normal responses to two stressors. One-half of the MBH is apparently inadequate for maintaining normal caloric homeostasis since unilateral MBH ablation resulted in normophagic, obese animals.

The results of unilateral ablation of MBH indicate that although there was some interference with ovulation, 87% of animals in this group had normal estrous cycles and ovulation. These observations suggest that one-half of the MBH is sufficient to maintain cyclic pituitary-ovarian function and that there is redundancy in the intact system. These results are also consistent with those that suggest redundancy in the bilateral preoptico-tuberal inputs to the MBH (41, 121, 191).

The finding that approximately 75% of the animals in the partial unilateral MBH group failed to ovulate indicates that these knife cuts were not innocuous. The data from those animals that failed to ovulate showed that all had demonstrated some irregularity in estrous cycles, with periods of either prolonged estrus or diestrus. On the basis of histological examination of lesions, there were no noticeable differences between those partial unilateral ablations that were compatible with ovulation and those that were not. It is possible, however, that damage to the median eminence, which is difficult to

assess, was responsible for the interruption of cycles and ovulation in some animals.

Although differences between experimental groups were not significant, the data suggest that the groups with partial and complete unilateral MBH-ablation developed approximately one-half of the preovulatory LH surge produced by the intact and sham-operated controls. This finding suggests that a quantitative relationship exists between the amount of MBH tissue present and the amplitude of the preovulatory surge of LH that is attainable. Thus, it appears that with one-half of the normal population of LRF-secreting neurons present, only 50% of the normal quantity of LRF is secreted into the pituitary portal circulation during the critical period of proestrus and that this evokes one-half of the normal preovulatory surge of LH. While it is held that the preoptico-tuberal system of nerve fibers that project to the MBH are responsible for LH release (17, 24, 36, 90, 121), the question as to whether this pathway has a bilateral (40, 66, 191) or ipsilateral (194) distribution in the MBH is controversial. The data from the present experiments are consistent with either pattern of distribution.

Although the preovulatory levels of LH observed in the hypothalamic-cut rats were approximately 50% of those in the control groups, ovulation occurred in the majority of the lesioned animals.

Therefore, these data suggest that there is considerable redundancy in the amount of LH released for ovulation. This indication of a redundancy in preovulatory LH levels is consistent with observations of others. Everett et al. (66), Turgeon and Barraclough (194) and Grieg and Weisz (85), found that 10-15% of the peak LH values attained during the proestrous surge is sufficient for complete ovulation.

Ovarian compensatory hypertrophy was maintained in animals with unilateral ablation, indicating that ablation of one-half of the MBH did not interfere with this feedback response to a reduction in circulating levels of ovarian steroids. Current evidence, derived primarily from steroid implantation and hypothalamic lesions, suggests that both the MBH and adenohypophysis are involved in the inhibitory feedback effects of ovarian steroids (90, 207). The present data do not bear on the question of site of feedback action, but they imply that one-half of the MBH is capable of supporting normal compensatory responses of the pituitary and ovary to decreased negative feedback. Complete MBH ablation results in ovarian atrophy (69, 70), presumably due to the elimination of LRF-secreting neurons located in this region (33) and, hence, to a marked deficit in LH release from the adenohypophysis.

The finding that 3 min immobilization stress produced a marked decrease in serum LH levels in the control groups following ovariectomy is in agreement with reports that immobilization inhibits LH

secretion in the proestrous female rat (59). The initial level of plasma LH appears to be important to the response, as animals with low initial LH levels have not demonstrated this decrement (59). In contrast with the above reports, Krulich and Illner (123) found that immobilization stress applied to intact male rats, with low initial LH levels, increases LH concentrations. The basis for this apparent sex difference is unknown. It is also unresolved as to how immobilization produces its effects on LH secretion, but it may act through such structures as the corticomedial amygdala (44, 126) or the hippocampus (196), both shown to exert inhibitory effects on gonadotropin secretion.

The finding that animals with complete or partial unilateral MBH ablation showed LH decrements to immobilization stress comparable with those of controls indicates that one-half of the MBH is sufficient to maintain the LH response to this stressor. Importantly, the non-stress LH levels in the ovariectomized, unilateral MBH-ablated rats were approximately 50% ($p < 0.01$) lower than the corresponding levels in controls. This reduction suggests that one-half of an MBH is capable of supporting only one-half of the normal post-ovariectomy rise in LH secretion. Presumably, as MBH tissue is eliminated, proportionately less LRF is secreted and, correspondingly, less LH is released from the adenohypophysis.

The present studies furnished somewhat ambiguous information regarding the effects of unilateral MBH ablation on GH secretion. Whereas rats with unilateral ablation had non-stress GH levels that were approximately 50% lower than those of intact controls in all three experiments in which such levels were assessed, there was not a similar difference between the experimental and sham-operated groups. It must be concluded, therefore, that sham operation and unilateral MBH ablation had comparable effects on GH secretion. In retrospect, it is unfortunate that the sham surgery involved placing the knife in the anterior hypothalamus. This may not have been an innocuous procedure. Despite this complication to interpretation, it appears that unilateral ablation of MBH was associated with an approximate 50% decrease in circulating GH levels on all three occasions when it was measured.

Since the original finding by Hetherington and Ranson (110) that lesions placed in the VMN in rats result in retarded growth, this hypothalamic area has been implicated in the control of growth and GH secretion. Extensive studies over the past three decades, involving electrical stimulation and lesioning techniques, have now convincingly demonstrated that the arcuate-VMN region is intimately involved in the control of GH secretion (18, 19, 20, 21, 73, 74, 133, 134, 135, 148). Operationally, it is presumed that nerve fibers from the VMN facilitate the release of GRF from the median eminence,

either directly or through the arcuate nuclei, which then traverses the pituitary-portal circulation to stimulate adenohypophysial somatotrophs to liberate GH. Accordingly, Martin (133) has referred to the MBH as the "final common pathway" in GH secretion. However, since the discovery of GIF, it has been proposed that there is a dual control system for GH secretion (139). Recent evidence suggests that GIF-secreting neurons are in part located in the preoptic area (5) and that these are involved in the stress-induced inhibition of GH secretion (14). Thus, the results of the present experiments, with the qualifications in interpretation discussed above, can be explained within the context of this postulated control system. Because resting levels of GH in the rats with unilateral ablation were approximately 50% of the corresponding levels in the intact controls ($p < 0.05$), it appears that a full complement of MBH GRF-secreting neurons is required to maintain normal resting GH levels. Although the unilateral MBH-ablated animals maintained lower resting GH levels than intact controls, they were able to attain body lengths and heart and kidney weights comparable to those of the intact and sham-operated groups, suggesting that one-half of the MBH is sufficient to maintain normal growth.

The group with partial unilateral MBH-ablation not only had GH levels that were lower than those of intact controls, but they showed reduced linear growth and lower body, kidney and heart weights

($p < 0.05$) than the intact and sham-operated controls. These results suggest that these lesions severely depressed both growth and GH secretion. Inspection of brain sections from individual rats showed that the majority of these animals had unilateral lesions that spared the anterior parts of the arcuate-VMN complex and surrounding tissue, while eliminating the posterolateral parts of this complex. This pattern of destruction may be significant in view of recent findings from Martin's laboratory, suggesting that afferents to the MBH that are inhibitory to GH secretion reach the arcuate-VMN complex via the anterior hypothalamic area (133, 134, 135). In this regard, Collu et al. (39) reported that extrahypothalamic structures inhibit the secretion of GH through anterior projections to MBH and postulated that posterolateral inputs are stimulatory to such secretion. Because the animals in the present studies with partial unilateral MBH ablation had lesions that generally spared the anterior hypothalamic region and because these rats demonstrated a relative deficiency of plasma GH, it is possible that facilitatory structures were removed while structures mediating inhibition were retained with these partial lesions.

A statistically significant diurnal variation in plasma GH was not demonstrated in any of the groups. This inability to detect a rhythm and the large variance observed are in agreement with the findings of Takahashi et al. (189). However, these investigators

noticed that the lowest plasma GH levels occurred at the period of light-dark transition. There was a similar tendency in the control groups of the present studies, but this was not a convincing pattern.

The inhibitory effects of ether and immobilization on plasma levels of GH in the two control groups are in agreement with other reports on the effect of stress in the rat (39, 53, 124, 168, 177, 189). Although the stress-induced decrements in the complete and partial unilateral MBH-ablated animals were generally less than those of the controls, due to the low resting GH levels of the former groups, the stress-induced inhibition of GH was not impaired. These observations suggest that one-half of the MBH is sufficient to maintain normal responses to both ether- and immobilization-induced GH stress. The amount of MBH tissue needed for this response is unknown. However, at least a portion of the MBH must be present to maintain a response to stress, because the inhibition of GH secretion is absent in animals with complete MBH ablation (53).

The pathways by which ether or immobilization inhibit GH secretion are unknown. Collu et al. (39) observed ether-induced GH responses after deafferentation, suggesting that this stressor acts directly on the MBH-pituitary unit. In contrast, others reported that this response was blocked in similarly deafferented animals (141, 142). Little is known concerning the structures or mechanisms involved in the inhibition of GH secretion by immobilization. Rice

and Critchlow (168) found that ablating the preoptic area blocked the normal response and resulted in a paradoxical increment in GH secretion, suggesting that this stressor may act through the preoptic area to produce its effects. Although it appears that stress-induced inhibition of GH secretion may act through the preoptic area to stimulate a release of GIF (5, 14) and thus inhibit GH secretion, this response may also reflect a decrease in GRF release.

With regard to pituitary-adrenal function, complete or partial unilateral MBH ablation were compatible with maintenance of the normal diurnal rhythm of resting plasma corticosterone levels, suggesting that the mechanisms responsible for the circadian rhythm in ACTH secretion were left intact. The temporal pattern of the diurnal changes in pituitary-adrenal function noted in these experiments was consistent with that described in other reports (94, 153, 155). However, the true peak, usually occurring between 1600 and 1800, was most likely missed due to the sampling times used.

The unilateral MBH knife cuts were also compatible with apparently normal corticosterone responses to ether and immobilization stress. Because changes in circulating levels of corticosterone were used as an index of changes in ACTH secretion, these results provide only limited information concerning the effects of these lesions on the release of ACTH. The data imply only that mechanisms regulating rhythmic and stress-induced changes in ACTH secretion

were intact and that the adrenal cortical responses were essentially normal. These results cannot be taken to indicate that ACTH secretion was quantitatively normal. A 50% reduction in ACTH release may not be detectable with the methods used.

Although the specific neural structures essential for the maintenance of the pituitary-adrenal circadian rhythm have not been fully revealed, much progress has been achieved. Using techniques for completely isolating the MBH by microsurgery or forebrain removal, investigators have found abolition of diurnal variations in plasma corticosterone levels (94, 153, 155). These findings suggest that connections to MBH are essential for this rhythm. It appears that nerve fibers projecting to the MBH from the anterior hypothalamic or preoptic area are indispensable in this regard (94, 144, 155). In this sense, the pituitary-adrenal system is similar to the LH system in that it depends on afferents to MBH from the anterior hypothalamic or preoptic area for normal rhythmicity (17, 89, 90, 121). Structures such as the fornix, hippocampus and septum are not essential for the corticosterone circadian rhythm (200, 201). The data from the present study suggest that input from the anterior hypothalamus-preoptic area to one-half of MBH is sufficient to cause a rhythm in adrenal cortical function that is quantitatively and temporally normal. Whether the lesions caused a decrease in ACTH or CRF secretion remains to be determined.

In the present studies, unilateral MBH-lesioned animals had stress-induced increments in plasma corticosterone comparable to those of controls. Ether-induced stimulation of ACTH secretion has been studied with both MBH deafferentation and forebrain ablation techniques, and it appears that MBH tissue dorsal to median eminence is not required for this response (84, 136). The persistence of the response to ether in the present studies suggests that approximately 50% of the terminals in the median eminence is adequate to maintain the pituitary-adrenal response to this stressor. Although somewhat diminished in amplitude, the pituitary-adrenal response to immobilization persists in rats in which the MBH has been isolated with the deafferentation technique (68, 155). As with ether, similar responses were observed in preparations with median eminence-pituitary islands (169). Again, it appears from the present data that approximately 50% of an intact median eminence is sufficient to maintain this neuroendocrine response. However, as discussed above, it cannot be inferred from these results that stress-induced ACTH secretion was quantitatively normal in these preparations.

The main effects of unilateral MBH knife cuts on food intake and obesity in the present study varied according to the type of lesion. Rats with complete unilateral MBH ablation showed normophagia, significant weight gain and obesity while maintaining normal linear growth. Animals with incomplete unilateral MBH ablation

demonstrated normophagia, decreased weight gain, no obesity and stunted growth.

The findings of excessive weight gain and obesity in the rats with unilateral MBH ablation are in agreement with several reports in which the VMN was unilaterally lesioned (137, 198) or separated from the lateral hypothalamic area by a knife cut (80). However, other investigators reported that neither unilateral VMN (110) nor unilateral lateral hypothalamic area (9) lesions affected food intake, weight gain or obesity. The basis for this difference in results is difficult to explain due to variations in techniques, feeding schedules and duration of observations, but it may be accounted for by the size of the VMN lesions. Larger lesions consistently produce more obesity than smaller lesions (18, 20, 21). Unilateral ventrolateral hypothalamic area lesions have been shown to cause transient aphagia (78, 81), also suggesting that unilateral hypothalamic lesions can disrupt control of food intake and obesity.

The magnitude of weight gain in the animals of the present study was comparable with that observed by Mayer and Barrnett in rats with unilateral lesions (137). This weight gain is seen in both normophagic (80, 137) and hyperphagic rats (137). Although the present study did not include rats with bilateral MBH lesions, it appears that animals with unilateral MBH ablation became less obese and show less weight gain than those studied previously in this

laboratory with bilateral MBH destruction (54) or cuts (156). Other investigators, comparing the effects of these lesions, have also found this to be the case (80, 137).

The present findings of obesity and normal body length in animals with unilateral MBH lesions, together with reports of obesity accompanying both increased (141, 142, 156) and decreased body length (18, 20, 21, 73), support the contention (18) that control of growth and obesity are subserved by different neuronal mechanisms having a common locus in the VMN. These mechanisms can apparently be dissociated.

While the animals with unilateral MBH ablation had normal linear growth and significantly decreased GH levels, these rats were normophagic yet obese. The latter two findings are consistent with prior results in both weanling and adult rats (18, 20, 21, 73). Bernardis and Frohman (18, 73) suggest that these effects may be attributed to altered metabolism, possibly due to interference with control of insulin secretion. They found that large VMN lesions caused hyperinsulinemia and altered lipid metabolism in rats, suggesting that insulin secretion may be influenced by the VMN, as is the case for GH secretion. Bernardis and Frohman postulate that these two hormonal control mechanisms are subserved by independent neuronal systems. In their view, neurons concerned with GH

do not appear to be due solely to a gross deficiency in circulating GH levels. These rats grew less than those with complete unilateral ablation, but GH levels were comparable in these two groups during each of several experiments.

SUMMARY AND CONCLUSIONS

These experiments were undertaken to determine whether there is functional redundancy in the bilateral representation of connections and neurons that comprise the medial basal hypothalamus. The experimental approach involved studying the effects of complete and partial unilateral MBH ablation on several MBH-mediated functions, including estrous cycles, ovulation and plasma LH levels, plasma GH levels and linear growth, plasma corticosterone levels, food intake and control of obesity.

The findings suggest that whereas one-half of the MBH is sufficient for maintaining some functions, it is inadequate for others. Bilateral MBH structures appear necessary to maintain normal circulating levels of LH and GH and for maintenance of the normal compensatory post-castration rise in LH. Unilateral MBH-lesioned animals maintained levels of the above hormones that were approximately 50% of those of controls. An intact MBH also appears essential for normal caloric homeostasis, because complete unilateral MBH ablation led to significant obesity without an increase in food intake. In so far as the lesioned animals manifested proestrous LH surges, ovulated, maintained ovarian compensatory hypertrophy, demonstrated a normal 24 hr pattern in plasma corticosterone levels, showed normal corticosterone, GH and LH responses

to stress, it appears that there is a redundancy of MBH tissue with respect to these neuroendocrine functions.

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