

COMPARISON OF THE METABOLIC AND BEHAVIORAL DISTURBANCES FOLLOWING
PARAVENTRICULAR- AND VENTROMEDIAL-HYPOTHALAMIC LESIONS

BY

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ABSTRACT

Lesions of the ventromedial hypothalamus (VMH) result in an obesity syndrome characterized by metabolic and behavioral disturbances. It has recently been indicated that damage to the paraventricular nucleus of the hypothalamus (PVH) also leads to changes characteristic of obesity. Although deficits following VMH lesions have been characterized extensively, less is known about the consequences of PVH damage. This thesis presents a series of experiments providing a detailed comparison of the two hypothalamic lesion syndromes.

Initially, to assess the basic features of the syndromes, rats underwent VMH, PVH, or sham lesions and were maintained ad libitum for 15 weeks on a series of test diets. Overall, lesion groups gained similar amounts of weight (significantly more than controls) and were equally hyperphagic. However, carcass analyses revealed that although both lesion groups had larger body fat compartments than controls, VMH rats were fatter than PVH animals. Similarly, although insulin levels in both lesion groups were elevated, only VMH rats had a significant hyperinsulinemia.

A defining feature of the VMH obesity is the development of certain disturbances of visceral secretion and excessive adipose stores even in the absence of hyperphagia. To assess whether the PVH obesity shares these characteristics, food intake of PVH and VMH rats were restricted postlesion to control body weights. First, gastric acid

secretion was measured to index lesion-induced changes in visceral secretion. VMH rats developed a persisting hypersecretion immediately postlesion; acid secretion levels of PVH rats were normal. In a second experiment, PVH and VMH rats were maintained at control weights for 28 days postlesion by restricted feeding of either a standard pellet or high fat diet. In both diet conditions, VMH rats became obese but PVH rats did not.

Finally, the effects of PVH and VMH lesions on behavioral reactivity to orosensory properties of food were assessed by comparing the sham feeding responses of PVH, VMH and control rats to liquid diets varying in palatability. Control animals increased sham feeding with ascending sucrose concentrations. VMH animals showed disproportionately large increases in consumption with increased sucrose. PVH animals showed sham feeding changes similar to VMH rats.

These data indicate similar effects of PVH and VMH lesions on behavioral measures; specifically, in normal, and sham, feeding. However, these two lesions produce different effects on metabolic and secretion measures. It is concluded that the etiologies of the two obesity are fundamentally different.

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LIST OF ABBREVIATIONS

C	centrigriade
cm	centimetre
DMN	dorsomedial hypothalamic nucleus
F	fornix
gms.	grams
H ⁺	hydrogen ion
HA	anterior hypothalamic nucleus
ip	intraperitoneal
kg	Kilogram
mamp	milliamperere
ml	milliliter
min	minute
mm	millimetre
OT	optic tract
PVH	paraventricular hypothalamus
PVN	paraventricular hypothalamic nucleus
RE	nucleus reuniens
μJ	microunits
μEq	microequivalents
VMH	ventromedial hypothalamus
VMN	ventromedial hypothalamic nucleus

CHAPTER 1. INTRODUCTION

Experimental investigations of feeding behavior, and related phenomena such as body weight maintenance and energy regulation, have attracted interest from a variety of disciplines. In physiological psychology, analysis of feeding behavior has concentrated on physiological mechanisms in the periphery and central nervous system which mediate eating. One approach adopted for these investigations has been to study the pathogenesis of feeding disorders produced by experimentally-induced damage to certain parts of the brain. One class of these feeding disorders is produced by damage to the hypothalamus, a structure within the diencephalon associated with homeostasis and vegetative function. Either anorexia (cessation of eating) or hyperphagia (excessive eating) can be produced by hypothalamic ablation depending on the locus of damage in the hypothalamus. These hypothalamic eating disorders have become model systems for physiological investigation of eating because it is believed that identification of the mechanisms producing the eating disturbances will provide insight into the central nervous system control of feeding behavior. The present thesis explores in detail the behavioral and physiological disturbances produced by damage to two hypothalamic sites, the ventromedial hypothalamus (VMH) and paraventricular hypothalamus (PVH).

In a series of experiments in the early 1940's, Hetherington and Ranson (1939, 1940, 1942a and 1942b) demonstrated that bilateral

electrolytic lesions of the VMH caused animals to overeat and become obese. Their findings indicated the VMH as a brain site involved in the control of feeding behavior. During the next four decades, the behavioral and physiological changes induced by VMH lesions were investigated extensively and the results of these analyses have been extended to substantiate various theories on the physiological control of feeding behavior and body weight (see Bray and York, 1979; Powley, Opsahl, Cox and Weingarten, 1980).

More recently, it has been demonstrated that damage to another site in the hypothalamus, the paraventricular nucleus of the hypothalamus (PVH), located just anterior and dorsal to the VMH, also produces overeating and increased body weight (Aravich and Scalfani, 1983; Leibowitz, Hammer and Chang, 1981; Scalfani and Aravich, 1983). These findings suggest the PVH as another brain locus participating in the regulation of feeding or as part of a neural circuit common with the VMH involved in the control of these functions. The discovery of a second hypothalamic site with effects on feeding and body weight similar to the VMH raises several questions regarding our present conceptualizations of the central nervous system control of feeding and body weight. For example, do the PVH and VMH represent two points in a common hypothalamic neural circuit mediating feeding and body weight control or do they operate via independent mechanisms? As well, how similar or different are the physiological mechanisms mediating the behavioral and physiological changes associated with damage to these areas? Answers to these questions require a detailed comparison of the

similarities and differences between the behavioral and physiological changes produced by damage to these two hypothalamic sites.

The present thesis was undertaken to provide such an analysis by comparing the behavioral and physiological changes following PVH and VMH lesions. The available literature on VMH-lesion effects is extensive. In contrast, little is known about the consequences of PVH lesions on either physiology or behavior. Thus, this thesis also serves to document the changes produced by damage to the PVH.

In the remainder of this introduction, the data on VMH lesion effects are reviewed selectively with the aim of characterizing the disturbances comprising the VMH syndrome and examining the pathogenesis of this syndrome. Next, the relevant literature on PVH lesion effects is summarized with special emphasis on identification of the critical information needed to further characterize this syndrome and to elucidate the pathogenesis of the PVH lesion syndrome.

I.1 THE VMH SYNDROME

Two classes of disturbances, behavioral and metabolic, are produced by VMH lesions. The characteristics of these dysfunctions are discussed in turn.

I.1.1 Behavioral Disturbances of the VMH Syndrome

The most pronounced behavioral difference between VMH lesion and normal animals is an elevation of the lesion animals' food intake, i.e., a hyperphagia. Correlated with this hyperphagia is a corresponding body weight increase. The hyperphagia and excessive

weight gain following VMH lesions possess several characteristics. First, hyperphagia and weight gain are apparent immediately postlesion. Some of the earliest descriptions of this syndrome documented that VMH rats took inordinately large meals immediately upon awakening from the anesthesia (Brobeck, Tepperman and Long, 1943). In fact, the extreme hyperphagia observed in the hours following surgery is often lethal and it has become customary to deprive VMH rats for about 24 hours after surgery.

A second characteristic is that the degree of hyperphagia and weight gain vary with time postlesion. Two phases are distinguished. The "dynamic phase" is the period of greatest hyperphagia and most rapid weight gain. During the dynamic phase, VMH rats consume as much as 2 to 3 times normal daily caloric intake (Corbit and Stellar, 1964). This elevated level of food intake is due primarily to an increased meal size (Brooks, Lockwood and Wiggins, 1946; Tepperman, Brobeck and Long, 1943), although a small increase in meal frequency, especially during the daylight hours, may also be observed (Becker and Kissileff, 1974). Gradually, the degree of hyperphagia and the rate of weight gain decrease and, when this occurs, the VMH lesion animal has entered the "static phase". In the static phase, VMH rats consume only slightly more food than normal, but their body weights remain at elevated levels (Brooks and Lambert, 1964). The decreased eating during the static phase does not represent a recovery of the disrupted feeding controls, since restricting the VMH rat's food intake during the static period initiates a second dynamic phase of hyperphagia and weight gain (Brooks and Lambert, 1964; Hoebel and Teitelbaum, 1966).

A third feature of the VMH lesion effects on hyperphagia and weight gain is the dependence of the magnitude of these disturbances on the diet upon which the rats are maintained. The degree of hyperphagia is influenced considerably by maintenance diet. For example, VMH rats maintained on a high fat diet may consume twice normal levels. This diet produces the largest body weight gains (Corbit and Stellar, 1964; Teitelbaum, 1955). On carbohydrate-rich diets, VMH rats are less hyperphagic and weight gain is less pronounced (Graff and Stellar, 1962; Teitelbaum, 1955). If the maintenance diet is adulterated with appropriate amounts of a bitter adulterant, such as quinine, the food intake of VMH lesion rats may drop sharply (Ferguson and Keesey, 1975; Kennedy, 1950; Miller, Bailey and Stevenson, 1950; Teitelbaum, 1955), and, in fact, may fall to normal levels (Ferguson and Keesey, 1975).

It has been established that the modulatory impact of different diets on VMH lesion-induced hyperphagia reflects a lesion-induced alteration in reactivity to sensory properties of foods, such as taste and texture. Changes in the texture of a diet, without affecting the taste or caloric value, affect the magnitude of hyperphagia. VMH rats maintained on powdered rat pellets evidence little or no hyperphagia (Corbit and Stellar, 1964). However, the same diet in pellet form, or mixed with nonnutritive mineral oil, supports a more dramatic hyperphagia (Carlisle and Stellar, 1962). This behavioral hyperreactivity to the sensory qualities of foods is a direct consequence of the VMH lesions and, contrary to several suggestions, is not a secondary consequence of an increased body weight. For example, Weingarten (1982) demonstrated the altered taste reactivity even in VMH

rats maintained at normal body weights. Thus, in the behavioral realm, three distinctive characteristics of VMH lesions are evident -- i) excessive level of eating, i.e., hyperphagia; ii) increased weight gain; and iii) an altered responsiveness to the sensory properties of food.

I.1.2 Metabolic Disturbances of the VMH Syndrome

VMH rats also exhibit metabolic dysfunctions. The most apparent effect is the obesity which develops postlesion. The definition of obesity is an excessive accumulation of body fat. The elevated body weights of VMH rats result almost exclusively from an increase in the size of the body fat compartment (Bernardis and Frohman, 1970; Holm, Hustvedt and Lovo, 1973; Hetherington and Weil, 1940; Keesey and Powley, 1975). The degree of obesity can be dramatic. VMH rats maintained on high fat diets may evidence fat compartments which constitute more than 50% of total body weight (Hetherington and Ranson, 1940; Kennedy, 1950; Montemurro and Stevenson, 1957). The increase in body fat results from an increase in the size of the fat cell, i.e., an adipocyte hypertrophy (Hirsch and Han, 1969).

A critical, and only more recently recognized, feature of VMH obesity is that its development is independent of hyperphagia. Although overeating enhances the degree of obesity, an elevation of fat stores occurs even when the food intake of the VMH rat is restricted to control levels (Cox and Powley, 1981; Han, 1967). In these studies, pair-feeding regimens are used to eliminate hyperphagia by feeding VMH rats only as much food as is eaten by a paired control animal. Normophagic VMH rats, however, still develop significantly greater

levels of carcass fat. This finding indicates that the development of the obesity in VMH lesion animals is not a consequence of hyperphagia, but rather, it is a direct effect of the VMH lesions.

VMH animals also display elevated levels of visceral secretion. For example, VMH rats have significantly higher levels of circulating plasma insulin (Frohman, 1970; Frohman and Bernardis, 1968; Hales and Kennedy, 1964) and gastric acid (Inoue and Bray, 1977; Powley and Opsahl, 1974; Ridley and Brooks, 1965; Weingarten and Powley, 1980). These changes are often apparent within hours or days of the production of the lesions (Berthoud and Jeanrenaud, 1977; Han and Frohman, 1970; Ridley and Brooks, 1965; Rohner et al., 1977; Steffens, Mogenson and Stevenson, 1972; Weingarten and Powley, 1980). As well, similar to the obesity, these secretion changes can be independent of hyperphagia. Even VMH rats prevented from hyperphagia and excessive weight gain by restricted feeding show elevated levels of plasma insulin (Han and Frohman, 1970; Gox and Powley, 1981) and gastric acid (Ridley and Brooks, 1965; Weingarten and Powley, 1980).

I.1.3 Pathogenesis of the VMH Syndrome

Traditionally, attempts to explain the pathogenesis of the VMH syndrome have assumed that VMH lesions induce some primary disturbance and that this essential disturbance leads, secondarily, to the development of other symptoms of the syndrome.

Two different hypotheses of this kind have been proposed. Initially, Brobeck and colleagues (1943, 1946) suggested that the lesion induced a primary behavioral change, the hyperphagia. It was

believed that the metabolic and physiological changes characteristic of the syndrome developed, secondarily, from the lesioned animal's overeating. This hypothesis was instrumental to the development of the influential view that the VMH was a "satiety center" which directly controlled the termination of eating. The subsequent demonstration that lesions of the lateral areas of the hypothalamus (LH) caused an animal to cease eating (Anand and Brobeck, 1951) led to the development of a "dual-center" model for the control of feeding which argued that the hypothalamus contained two centers controlling eating: a VMH satiety center controlling meal termination and a LH feeding center responsible for meal initiation. This model of feeding control was readily accepted by researchers and became a dominant view to explain brain control of motivated behavior (e.g., Stellar, 1954).

In the late 1960's, data began to accumulate demonstrating the inadequacy of the view that hyperphagia was the primary cause of the syndrome. In a series of studies, Han and colleagues (1968, 1970) found that elimination of the assumed primary disturbance, i.e., the hyperphagia, did not prevent the development of the assumed secondary disturbances. Specifically, they demonstrated that hyperinsulinemia and obesity (i.e., higher levels of body fat) developed even in VMH rats which were prevented from hyperphagia by being pair-fed with intact controls. This observation has been replicated often. It is now clear that food-restricted and normal-weight VMH rats still exhibit obesity and other secretion disturbances (see Bray and York, 1979 and Powley et al., 1980 for review). The implication from these data is that hyperphagia cannot be the primary cause of the syndrome.

Later analyses suggested that while primary changes in satiety did not cause the syndrome, a primary lesion-induced alteration in visceral secretion mediated by the vagus or, more generally, by the autonomic nervous system, represented the essential disturbance leading to the development of the syndrome. This idea was suggested mainly by the experimental finding that bilateral subdiaphragmatic vagotomy reversed the hyperphagia and obesity of VMH rats (Powley and Opsahl, 1974; Inoue and Bray, 1977) and blocked the development of the syndrome if vagotomy was performed prior to the VMH lesions (Eng, Gold and Sawchenko, 1978; Rowland and Engle, 1978). These data led Powley (1977) to suggest that increased parasympathetic tone on the viscera was the primary causal factor leading to the development of the syndrome. Others have disagreed with Powley on the exact nature of the physiological changes producing the syndrome. For example, Bray and York (1981) suggested that a lesion-induced depression of sympathetic tone on the viscera resulted in the behavioral and physiological differences of the VMH syndrome. In spite of this controversy, it is now generally believed that a lesion-induced dysfunction in autonomically-mediated (whether parasympathetic or sympathetic) visceral secretion represents the primary causal factor in the development of the VMH syndrome.

In summary, VMH lesions induce a set of behavioral and metabolic disturbances. The behavioral disturbances include hyperphagia, weight gain and an over-responsiveness to sensory qualities of food. The metabolic ones include enhanced levels of body fat (obesity) and

elevations of autonomically-controlled visceral secretions manifest as hyperinsulinemia and gastric hyperacidity.

I.2 HISTORY OF THE PVH SYNDROME

A possible role for the PVH in the control of food intake or body weight was suggested more than 40 years ago by Heinbeck, White and Rolf (1944) who demonstrated overeating and body weight increases in dogs after PVH lesions. This finding was largely ignored, however, because of the more influential report of Hetherington (1944) who failed to observe any eating or weight changes in rats with lesions of the PVH. In fact, in a study aimed directly to localize hypothalamic sites involved in the control of feeding and body weight, Hetherington (1944) wrote that "...cell groups rostral and dorsal to the ventromedial hypothalamic nuclei (the site of the PVH) make little if any contribution to the regulation of fat metabolism", and that "...two of the animals underwent complete elimination of the paraventricular nuclei without becoming fat" (p. 40). The consequence of this emphatic statement was to lead researchers for about 30 years to ignore a possible role for the PVH in the control of feeding and body weight.

Several experimental findings in the 1970's led investigators to reassess the role of the PVH as a hypothalamic site for feeding control. First, it was found that lesions restricted to the ventromedial hypothalamic nucleus (the presumed site of the VMH syndrome) did not produce hyperphagia or weight gain (Gold, 1973). Therefore, it became apparent that other hypothalamic sites located within the vicinity of the VMH must be involved in hyperphagia.

Further mapping studies revealed, in fact, that lesions in the area of the PVH, which is situated rostral to the ventromedial nucleus, were effective in producing hyperphagia and weight gain (Gold, 1973).

The results of several hypothalamic knife-cut studies further implicated the PVH in feeding regulation. By making parasagittal or coronal knife-cuts in the medial hypothalamic area, it was shown that a longitudinal fibre system coursing through the medial hypothalamus was involved in the control of feeding. These studies also demonstrated that the effectiveness of the hypothalamic knife-cuts in producing hyperphagia and weight gain was dependent upon their location relative to the PVH. Parasagittal knife-cuts just lateral to the PVH produce a more profound increase in food intake and body weight than cuts posterior to the PVH (Gold, Jones, Sawchenko and Kapatos, 1977; Sclafani and Berner, 1977). Similarly, knife-cuts made in the coronal plane lost their effectiveness once the cuts were placed anterior to the PVH (Gold, Jones, Sawchenko and Kapatos, 1977). Based on these data, it was proposed that the hypothalamic fibre system controlling eating or body weight originated in, or coursed through the vicinity of, the PVH. Thus, Gold and his associates (1977) stated explicitly that the PVH could be the critical site mediating the hypothalamic knife-cut hyperphagia effects.

Anatomical studies in the early 1980's showing connections of the PVH to other brain loci also reinforced the idea of an involvement of the PVH in the characteristics generally associated with the VMH syndrome. Direct neural connections between the PVH and autonomic ganglia, such as the dorsal motor nucleus of the vagus, were shown and

led to suggestions that the PVH might exert functional control over the vagus nerve (Sawchenko and Swanson, 1981; Swanson and Kuypers, 1980; Swanson and Sawchenko, 1980). Since a change in vagally-mediated visceral responses was widely believed to be involved in the development of the VMH syndrome, the close anatomical links of the PVH to the vagus suggested a potential role for this brain site as well.

Neuropharmacological studies also implicated the PVH in the control of feeding. It was known that hypothalamic injections of norepinephrine stimulated feeding in sated rats and enhanced feeding in hungry rats (Grossman, 1964; Leibowitz, 1976). Mapping studies demonstrated that the PVH was the most sensitive site producing these effects (Leibowitz, 1980).

Finally, in the early 1980's, the conclusive experiments were done. These studies demonstrated that bilateral lesions of the PVH did increase food intake and body weight in adult male and female rats (Aravich and Sclafani, 1983; Leibowitz, Hammer and Chang, 1981; Sclafani and Aravich, 1983).

I.3 THE PVH OBESITY SYNDROME

Identification of food intake and body weight changes following PVH lesions are relatively new findings. There is no consensus about the range or type of behavioral and metabolic disturbances following these lesions. Similarly, the pathogenesis of the PVH syndrome is unclear. Although some have suggested that PVH lesions induce a similar syndrome as VMH lesions (Aravich and Sclafani, 1983), the paucity of data on this issue render such a conclusion unwarranted.

The available data on PVH lesion effects are discussed below and the information needed to evaluate the idea that PVH and VMH lesion syndromes are essentially similar is identified.

I.3.1 Behavioral Disturbances of the PVH Syndrome

PVH lesion rats increase their daily food intake and gain weight more rapidly than control animals (Aravich and Sclafani, 1983; Leibowitz et al., 1981; Sclafani and Aravich, 1983). In certain respects, these changes resemble the hyperphagia and weight gain of VMH lesion rats. First, the onset of these changes is rapid after lesions (Aravich and Sclafani, 1983). Second, as with every obesity, distinct dynamic and static phases can be distinguished; the rate of weight gain following PVH lesions gradually decreases with time postlesion and eventually becomes stable (Aravich and Sclafani, 1983). Finally, the magnitudes of hyperphagia and weight gain of PVH rats appear to be affected by maintenance diet. PVH rats overconsume ordinary laboratory diets, but adulteration of the diet with small amounts of a bitter substance, such as quinine or sucrose octaacetate, normalizes intake (Aravich and Sclafani, 1983). In contrast, on more palatable food, such as dextrose or high fat diets, PVH rats display hyperphagia (Aravich and Sclafani, 1983). These changes, at a superficial level, are reminiscent of VMH lesion rats.

Although these findings suggest similarities in some behavioral elements of the PVH and VMH lesion syndrome, several further comparisons are needed to establish that these two lesions produce the same behavioral disturbances. First, a direct comparison of food

intake and body weight changes in PVH and VMH rats is necessary in a single study in one laboratory. Otherwise, reported differences between the effects produced by lesions of these two sites may actually represent idiosyncracies of the procedures and techniques in different laboratories.

As well, another required behavioral comparison involves examination of the heightened behavioral reactivity to sensory qualities of food. This disturbance is a defining trait of the VMH syndrome. It is unclear whether PVH lesions also produce a similar disturbance. Although PVH rats exhibit fluctuations in hyperphagia dependent on diet (Aravich and Sclafani, 1983), this may not reflect an altered responsiveness to sensory properties of food in PVH rats, as it is with VMH animals. The data showing diet dependency in PVH rats were obtained in obese PVH rats (Aravich and Sclafani, 1983). The dependence of hyperphagia on diet in an obese group may reflect a secondary consequence of an already-developed obesity.

Two procedures must be used to ascertain whether PVH rats also exhibit heightened behavioral reactivity to sensory qualities of food. First, an experimental preparation must be used that establishes food intake changes when only oral sensory factors are operative. Second, such an experiment must eliminate the possible confound of body weight increases as mediators of the behavioral change. As indicated earlier, a sham feeding preparation adapted by Weingarten (1982) can be used to illustrate the hyperactivity to sensory aspects of food in VMH rats. In a sham feeding preparation, animals ingest liquid diet orally and the ingested food passes through the oropharyngeal area and drains out

of the stomach via a fistula. Thus, postabsorptive consequences of food intake are eliminated. In addition, testing for behavioral reactivity can take place under controlled body weight conditions so that any observed differences in sham feed intake between normal-weight lesion animals and controls cannot reflect a secondary consequence of increased body weight levels in lesion animals. These experiments are reported in Chapter 3 and 4.

1.3.2 Metabolic Disturbances of the PVH Syndrome

The physiological and metabolic changes following PVH lesions are poorly characterized. For instance, although percentage body fat is the defining characteristic of obesity, there is, in fact, no documentation of an elevated body fat level in PVH rats. There is one report implying this finding (Aravich and Sclafani, 1983), but this inference is based on Lee Index, an indirect calculation of body fat which may be inaccurate (Stephens, 1980).

Changes in visceral secretion following PVH lesions are also poorly characterized. For example, the available data on insulin secretion changes following PVH lesions are controversial. Some studies report elevated basal insulin levels in obese PVH rats (Aravich and Sclafani, 1982; Steves and Lorden, 1982). Other studies (conducted ironically by the same researchers) yield opposite results (Aravich and Sclafani, 1983; Steves and Lorden, 1984). The nature of this discrepancy is unclear. A possible difference between PVH and VMH lesion effects on insulin secretion is indicated by the finding that weanling rats with PVH lesions show normal basal insulin levels (Bernardis, 1984) whereas

weanling rats with VMH lesions exhibit a marked basal hyperinsulinemia (Frohman and Bernardis, 1968). The nature or origin of these discrepancies is unclear but these results indicate, at a minimum, that hyperinsulinemia might not be as strongly associated with PVH lesions as it is with VMH lesions.

Equally, there is no consensus about the effects of PVH lesions on another visceral secretion, gastric acid. Although there is a single report of the lack of effect of PVH lesions on basal acid secretion in dogs (Davis, Brooks and Steckel, 1968), no other data exist on changes in acid secretion following PVH lesions. Levels of basal acid secretion provide an index of vagal tone on the viscera since basal acid levels in the rat depend so heavily on vagal input. An increase in vagal tone on the visceral organs is implicated as a mechanism involved in the development of the VMH syndrome. Any suggestion of a common mechanism of the PVH and VMH syndromes must indicate whether PVH lesions also produce similar changes.

1.3.3 Pathogenesis of the PVH syndrome

Aravich and Sclafani (1983) hypothesized that the PVH and VMH syndromes shared a common etiology. Specifically, they hypothesized that both syndromes resulted from damage to a common neural substrate located in the anteromedial hypothalamus. This view is difficult to assess since several critical pieces of information must be collected before any hypothesis like this can be entertained. First, the behavioral and physiological disturbances of PVH animals must be characterized. The importance of these disturbances in the development

of the syndrome must be identified. Obesities are classified into two types (Mayer and Thomas, 1967). A "regulatory" obesity is one in which a primary disturbance in the regulation of feeding causes an excessive level of intake which leads, secondarily, to an increased body fat store. A "metabolic" obesity is one in which a primary metabolic disturbance leads to excessive fat levels; the hyperphagia characteristic of these latter obesities is viewed as secondary to the primary metabolic dysfunction. Classification of an obesity into a regulatory or metabolic type involves examination of physiological and body compartment changes in animals prevented from hyperphagia and thus maintained at control body weight levels. The obesity syndrome induced by VMH lesions is of the metabolic type as changes such as enhanced fat stores (Han, 1968; Cox and Powley, 1981) and elevated visceral secretion (Han and Frohman, 1972; Weingarten and Powley, 1980) develop even in the absence of hyperphagia or weight gain. The classification of the PVH obesity is unclear. Therefore, examinations of physiological and body compartment changes in PVH rats prevented from hyperphagia and maintained at control body weight levels are crucial in revealing the characteristics and etiology of the syndrome.

In summary, bilateral lesions of the PVH have been established recently as producing enhanced food intake and weight gain. It is unclear whether PVH lesions produce an obesity syndrome similar to the classically-defined VMH syndrome. The major difficulty is the lack of understanding of the behavioral and physiological changes following PVH lesions. VMH lesions produce a set of disturbances including hyperphagia, increased weight, overresponsiveness to sensory qualities

of food, obesity (i.e., elevated percent carcass fat), and visceral hypersecretion. In contrast, the only characterized disturbances following PVH lesions are hyperphagia and weight gain. Whether PVH lesions produce other indices of the VMH syndrome is unclear. The studies presented in this thesis address these issues by:

- i) direct comparisons of behavioral and physiological changes in PVH and VMH rats in order to evaluate the extent of similarity of the two hypothalamic syndromes (Chapter 3),
- ii) using restricted feeding paradigms to assess the relative importance of behavioral and metabolic changes in the development of the two lesion-induced obesities (Chapter 4),
- iii) using a sham feeding procedure to document whether PVH lesions also produce a dysfunction in the reactivity to stimulus qualities of food (Chapter 5).

CHAPTER II. GENERAL METHODS

Experimental Animals

Male Long-Evans hooded rats, approximately 3 to 4 month old, were used as subjects. The rats were bred in the McMaster Psychology Department colony from breeding stock obtained from Blue Spruce Farms (Altamont, New York). Animals were housed individually in hanging wire-mesh cages in an animal room maintained on a 16:8 hr light:dark cycle, and at 25°C. Water was available continuously throughout the experiments and food was provided according to the experimental protocols.

Hypothalamic Lesions.

All rats underwent either bilateral hypothalamic lesions or sham surgery. Prior to surgery, rats were anesthetized with sodium pentobarbital (Somnotol, 45 mg/kg; ip). They were then mounted into a Kopf stereotaxic instrument with the incisor bar adjusted 3.0 mm below the horizontal zero so that the skull was positioned horizontally. A 1.5 cm midline cut was made in the scalp. Depending upon the coordinates of the lesions, two 1 mm diameter holes on the surface of the skull were drilled and a stainless steel electrode (a #00 insect pin), coated with epoxylite except for 0.4 mm at the tip, was lowered into the brain. The coordinates for VMH lesions were: 2.3 mm behind bregma; 0.6 mm lateral to the midline; and 8.5 mm below the surface of

the skull. After lowering the electrode, VMH lesions were made by passing a 1.0 mamp anodal direct current for 17 seconds. A tail cathode served as the ground. For PVH lesions, the electrode was lowered to: 1.8 mm posterior to bregma; 0.2 mm lateral to the midline and 7.2 mm below the surface of the skull. A 1.0 mamp anodal direct current was passed for 12 seconds. After lesions, the holes on the skull were filled with bone wax, and the scalp was closed with wound clips. For the sham surgery, rats were anesthetized, the scalp exposed, holes drilled, but no electrodes were lowered into the brain.

Gastric Cannula Implantation

Chronic gastric cannulae were implanted in rats used in gastric acid collection (Chapter 4) and sham feeding (Chapter 5) studies. Details of cannula design have been described by Weingarten and Powley (1980a). Briefly, the gastric cannula consisted of an 11 mm long stainless steel tube (8.5 mm outside diameter X 7.9 mm inside diameter) flanged at both ends. A 2 cm X 2 cm disc of Marlex mesh was cemented to the shaft of the cannula. This disc of mesh anchored the cannula in the abdominal cavity. The inner wall of the cannula was tapped to accept a removable screw. During sham feeding or collection of gastric acid, the screw was removed and replaced by a 15 cm long drainage tube that screwed into the cannula shaft.

To implant the cannula, rats were food deprived for 24 hours prior to surgery to ensure a clean gastrointestinal tract. The rat was anesthetized with sodium pentobarbital (Somnotol, 45 mg/kg; ip). The abdominal area was shaved and cleaned with 70% alcohol. A 2-3cm

midline incision was made starting just below the sternum, and the stomach was gently lifted out of the abdomen. Two concentric purse string sutures (using 5-0 silk) were sewn into the wall of forestomach. A small cut was made in the area encircled by these sutures. One end of the gastric cannula was tied into the stomach with these sutures. A scalpel cut was then made through the left abdominal area, and the free end of the cannula was pulled through this cut. A second disc of mesh was forced onto the cannula shaft and placed between the body wall and skin to further anchor the cannula. The cannula was pulled through the skin and, finally, secured in place by tightening a purse string suture placed in the skin surrounding the skin incision. The abdominal wall was closed with interrupted sutures of 3-0 catgut and the skin was closed with wound clips.

Irritability Tests (Chapter 3)

The degree of irritability was assessed using a four category test described by Paxinos (1973). These categories were (1) biting reaction to presentation of a pencil at the top of the open cage; (2) biting response to appearance of a gloved hand placed into the cage; (3) biting reaction to the gloved hand pushing the rat against the cage wall; and (4) the vocal response during the preceding tests. The scales of irritability in these tests were scored as follows: 0 for no response; 1 for mild reaction; and 2 for intensive response. The irritability score (0-8) was computed by summing the score on each test.

Apparatus for Gastric Acid collection and Sham Feeding (Chapters 4 & 5)

Gastric acid collection and sham feeding tests took place in a custom designed Plexiglas cages (10 cm wide x 20 cm long x 10 cm high) which were mounted on 15 cm high stilts. The floor of these cages was constructed of stainless steel rods; the center rods were separated by 1.6 cm to allow a collecting tube to pass through the floor. A 2.5 cm diameter hole was located on the front wall of the cage so that a graduated cylinder could be attached to the outside of the cage and, when it was, its spout protruded into the cage.

Testing Procedures for Gastric Acid Secretion (Chapter 4)

Basal levels of gastric acid secretion were measured in PVH, VMH and control rats; these data are reported in Chapter 4. Animals were food deprived for 17 hours prior to acid collection. To collect gastric acid, the rat was removed from its home cage, its cannula opened and residual stomach contents were removed by saline lavage. Rats were then placed into the testing cage. A collecting tube was connected to the gastric cannula. The gastric juice drained by gravity flow down the collecting tube and collected into a 5 ml test tube force fit onto the end of the collecting tube.

Testing Procedures for Sham Feeding (Chapter 5)

Animals were tested after 3 hours of food deprivation. To establish this deprivation, three hours prior to a sham feeding test, the pellet food was removed from animals' home cages and they were allowed to consume an evaporated milk-based liquid diet for 10 minutes.

At the end of the 3 hours, the rats were removed from their home cages, and their cannulae were opened by removing the screws which normally kept them closed. Stomachs were washed with warm tap water to remove stomach contents. A collecting tube was screwed into the cannula shaft so that any liquid diet ingested by the rats during the test period would drain freely out of stomach. The rats were then placed into test cages, and a graduated cylinder containing a sucrose solution of a given concentration was attached to the test cage.

Sacrifice

At the end of each experiment, rats were anesthetized with sodium pentobarbital (Somnotol, 65mg/kg; ip) and sacrificed. Depending on the nature of the study, several procedures were followed:

(i) Blood Collection

Five ml of blood were drawn directly from the heart into a syringe heparinized with .02 ml of 100 U/ml heparin. Blood samples were centrifuged and the plasma removed and stored at -18°C .

(ii) Perfusion :

Rats were perfused intracardially with physiological saline followed by 10% buffered formalin. The brains of lesion rats were removed and stored in 10% formalin for later histological examinations.

(iii) Preparation of Carcass

To prepare the carcass for subsequent carcass fat analysis, the gastrointestinal tract of the perfused rat was cleaned, the

head and tail removed, and the carcasses stored at -18°C .

(iv) Physiological Measurements

Plasma glucose and insulin assays were performed by Dr. Tom J. McDonald at the University of Western Ontario, University Hospital, Department of Endocrinology, according to procedures detailed elsewhere (Brown et al., 1975).

(v) Carcass Fat Determination

For body composition analysis, the carcasses were cut, weighed and dessicated at 60°C . The difference in weight between the original and dessicated carcass weight provided the estimate of percentage body water. Body fat was calculated using a regression equation reported and validated by Cox and Powley (1984): percentage of body fat = percentage of body water $\times -1.272 + 95.963$.

(vi) Histology

The brains of lesion rats were frozen and sectioned in the coronal plane at 40 μm . One out of every three sections through the lesions was mounted. Slides were stained with luxol fast blue and cresyl violet for fiber tracts and cell bodies respectively.

Data Analysis

Data were analyzed with analyses of variance. A .05 probability level was considered statistically significant. Pair-wise comparisons, where justified, were made using the Studentized range statistic (q) and evaluated according to the Newman-Keuls procedure.

CHAPTER III. EFFECTS OF PVH AND VMH LESIONS ON FOOD INTAKE, BODY WEIGHT, IRRITABILITY, AND METABOLIC PROFILE IN RATS MAINTAINED ON AD LIBITUM FEEDING

Although lesions of the paraventricular hypothalamus (PVH) have been described as increasing food intake and body weight in rats (Aravich and Sclafani, 1983; Leibowitz, Hammer, and Chang, 1981; Sclafani and Aravich, 1983), the effects of PVH lesions have not been systematically compared with those induced by lesions of the ventromedial hypothalamus (VMH). As a first step in the comparison of these two hypothalamic obesity syndromes, characteristics of VMH and PVH rats were compared with special reference to some of the indices (identified before) which define the VMH syndrome.

METHODS

Experimental Procedure

Initially, 37 male Long-Evans hooded rats were weighed and assigned to either VMH-lesion (N=11), PVH-lesion (N=16), or control (N=10) groups such that the mean body weight and variance of the three groups would be equivalent at surgery. Rats underwent stereotaxic surgery for hypothalamic or sham lesions. After surgery, rats were deprived of food for 24 hours. For the next 110 days, subjects were maintained ad libitum on a series of test diets including Purina rat

pellets, Purina rat chow powder, mash and high fat diets. The composition of these foods and caloric contents are presented in Table 1. Subjects were given ad libitum access to these diets in the following sequence: Purina rat pellets (day 2-3), Purina rat chow powder (day 4-30), mash (day 31-50), high fat (day 51-70), mash (day 71-90), high fat (day 91-110). Body weights and 48-hour food intakes were recorded every second day. After day 110, all subjects were maintained ad libitum on the high fat diet for three days. On each of these days, subjects underwent the irritability testing. Then, rats were fasted for 24 hours and sacrificed according to the procedure detailed before.

RESULTS

Histology

Five of the original 11 VMH lesion rats were selected into the final VMH group and 7 of the original 16 PVH lesion rats constituted the final PVH group. All statistical analyses were based on data obtained from these animals and 10 control rats.

Animals selected into the PVH group met the criterion of bilateral destruction of the entire paraventricular hypothalamic nuclei (PVN). These lesions were centered on the caudal part of this nucleus. The lesions were considered to be the area of necrotic tissue (or cavity) plus the adjacent circling cell-free zone. The lesions generally extended beyond the PVN to include parts of the anterior hypothalamic nucleus, anteromedial aspects of dorsomedial hypothalamic nucleus,

<u>Diet</u>	<u>Composition</u>	<u>Kcal Value</u>	<u>Days used</u>
Pellets	-	3.61 Kcal/g	1-3
Powder	-	3.61 Kcal/g	4-30
Mash	65% Water 35% Powder	1.26 Kcal/g	31-50 71-90
High Fat	67% Powder 33% Crisco Oil	5.50 Kcal/g	51-70 91-110

Table 1. Composition and caloric content of the diets used in the ad libitum study.

nucleus reuniens of the thalamus and zona incerta. Except in one case, the ventromedial hypothalamic areas were completely spared. In the one animal with some VMH damage, the lesions encroached on the dorsal tip of the ventromedial hypothalamic nucleus. Figure 1 reconstructs the lesion damage placements of two PVH-lesion rats.

To be selected into the VMH group, the criterion was bilateral lesions centered on the ventromedial hypothalamus destroying all of the ventromedial hypothalamic nucleus (VMN). The lesions generally began in the anterior hypothalamic area and continued throughout the entire extent of the ventromedial nucleus. The lesions extended from the third ventricle laterally to the fornix and from the dorsomedial hypothalamic nucleus to the base of the brain (see Figure 2). No rat selected to the VMH group sustained damage to the PVN.

Postlesion Body Weight Changes

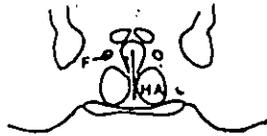
The body weight of the PVH, VMH, and control groups were similar at surgery, $F(2,19) = 2.32, p > .05$. The cumulative body weight gains of PVH, VMH and control rats postlesion are illustrated in the upper panel of Figure 3 and summarized in Table 2. On the initial test diet, powder, VMH rats gained weight rapidly so that, by the end of the powder period, they weighted significantly more than control, $q_3 = 5.45, p < .01$, and PVH, $q_2 = 3.85, p < .05$, rats. Although PVH rats gained 26% more weight than controls, this difference was not significant, $q_2 = 1.60, p > .05$. On the first exposure to mash, both VMH, $q_3 = 5.03, p < .01$, and PVH, $q_2 = 3.00, p < .05$, rats gained more weight than controls; VMH and PVH animals gained similar amounts of weight, $q_2 = 2.04, p >$

Figure 1. Reconstruction of two PVH lesions superimposed on frontal diagrams modified from the Konig and Klippel (1963) atlas. Structures are labeled in the left column, under "median control" animal. Numbers in the leftmost column indicate anterior-posterior level of each section. Various parameters of these individual animals are also presented. The center column reconstructs lesions for the PVH rat with the greatest body weight at sacrifice. The right column illustrates the lesions of the PVH rat median in final weight. The lesions were considered to be the area of necrotic tissue (or cavity) plus the adjacent circling cell-free zone.

(abbreviations: DMN, dorsomedial hypothalamic nucleus; F, fornix; HA, anterior hypothalamic nucleus; OT, optic tract; PVN, paraventricular hypothalamic nucleus; RE, nucleus reuniens; VMN, ventromedial hypothalamic nucleus.)

	MEDIAN CONTROL	LARGEST PVH	MEDIAN PVH
FINAL BODY WT.(g)	532	822	735
CARCASS FAT (%)	26.8	47.5	46.6
PLASMA INSULIN (uU/ml)	208	248	196

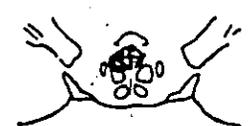
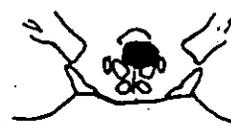
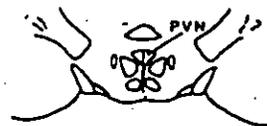
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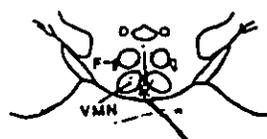
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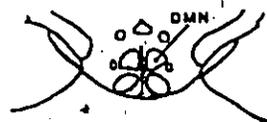
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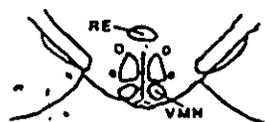


Figure 2. Schematic representations of two VMH lesions. Legend and abbreviations as in Figure 1.

	MEDIAN CONTROL	LARGEST VMH	MEDIAN VMH
FINAL BODY WT.(g)	532	842	790
CARCASS FAT (%)	26.8	53.8	52.4
PLASMA INSULIN (uU/ml)	208	557	1127

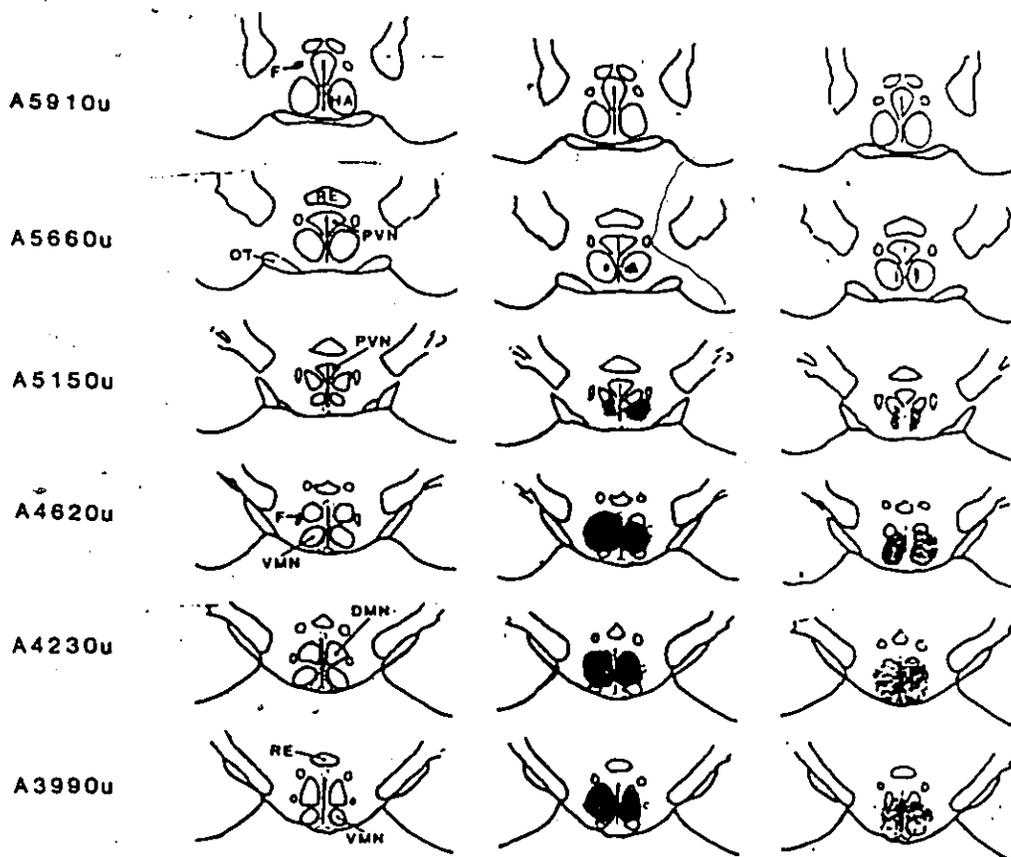
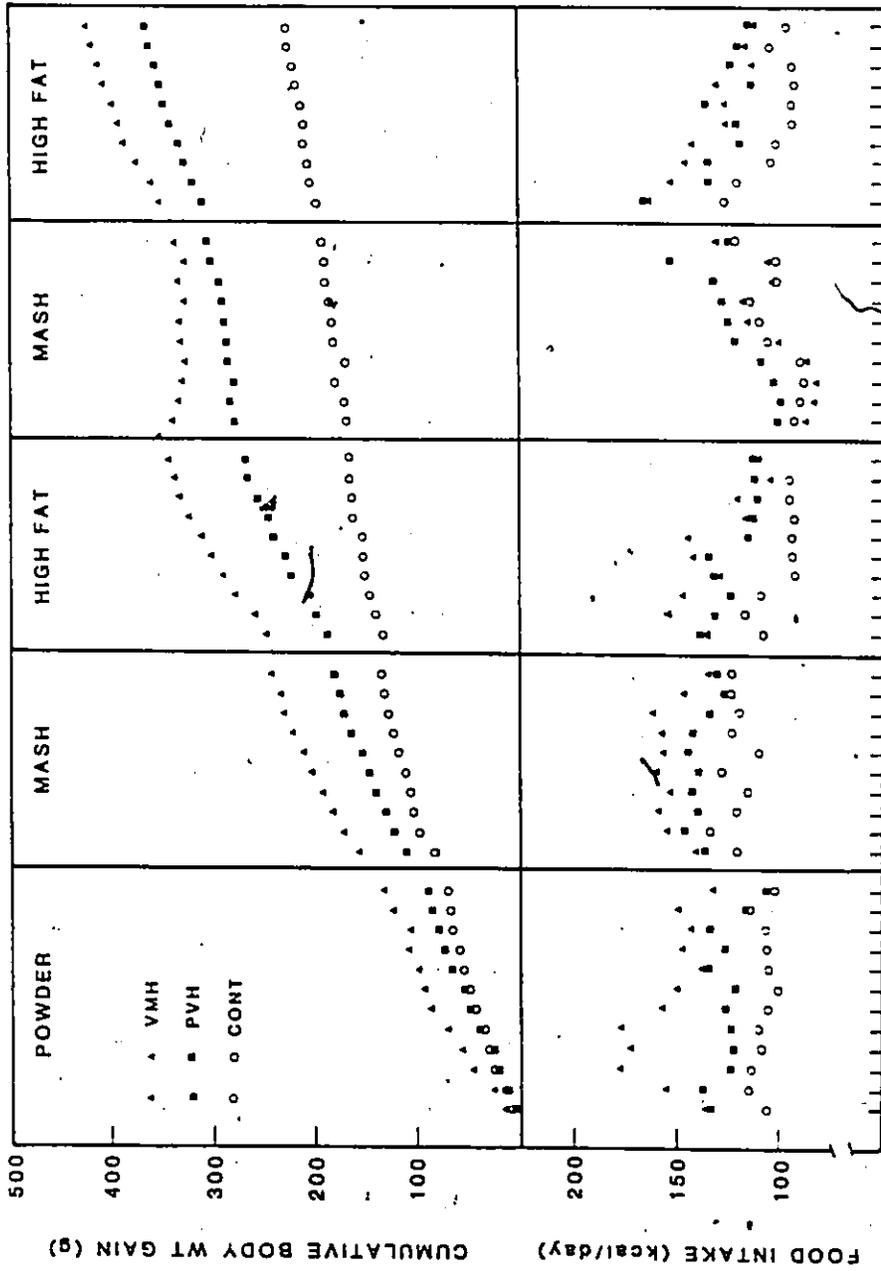


Figure 3. Upper panels -- Group mean cumulative body weight gain in VMH (N=5), PVH (N=7), and Control (N=10) rats maintained ad libitum on diets shown. Lower panels -- Group mean daily caloric intakes during the various phases of the experiment.



CONSECUTIVE 2-DAY BLOCKS

GROUP	N	DIET				
		Powder	Mash	High Fat	Mash	High Fat
Control	10	71.3 ± 6	67.6 ± 5.4	39.7 ± 5.9	29.8 ± 2.3	35.4 ± 5.0
PVH-lesion	7	90.1 ± 11.4	91.6 ± 4.9 ^{a*}	86.6 ± 7.3 ^a	42.3 ± 4.9	68.1 ± 5.2 ^a
VMH-lesion	5	135.2 ± 21.2 ^{a, b*}	128 ± 2.5 ^{a, b}	100.8 ± 11.7 ^a	-4.2 ± 4.7 ^{a, b*}	83.6 ± 15.8 ^a

Table 2. Mean (\pm SEM) body weight gained (in gms) by PVH, VMH and control groups during each of the diet periods. (a = significantly different from control at .01 or .05 (a*) level; b = significantly different from control at the .01 or .05 (b*) level.)

.05. On high fat, VMH, $q_3 = 7.67$, $p < .01$, and PVH, $q_2 = 5.93$, $p < .01$, rats gained more weight than controls, and the weight gains of the two lesion groups were similar, $q_2 = 1.74$, $p > .05$. In the second mash period, PVH and control rats gained similar amounts of weight, $q_2 = 1.91$, $p > .05$, and VMH animals showed significant weight losses compared to PVH, $q_3 = 6.98$, $p < .01$, and control, $q_2 = 5.07$, $p < .01$, rats. When returned to high fat, both VMH, $q_3 = 5.88$, $p < .01$, and PVH, $q_2 = 3.65$, $p < .05$, groups significantly outgained controls. Over the entire 110 day period, both VMH, $q_3 = 8.35$, $p < .01$, and PVH, $q_2 = 6.90$, $p < .01$, rats gained significantly more weight than controls, and the weight gains of the two lesion groups were similar, $q_2 = 1.44$, $p > .05$.

Postlesion Food Intake

The mean daily caloric intakes of the three groups are shown in lower panel of Figure 3 and summarized in Table 3. On powder, the daily intake of VMH rats exceeded control, $q_3 = 6.06$, $p < .01$, and PVH, $q_2 = 3.35$, $p < .05$, values. Although the average daily intake of PVH rats was 18% greater than controls, this difference was not significant, $q_2 = 2.71$, $p > .05$. On the initial exposure to mash, both VMH, $q_3 = 5.49$, $p < .01$, and PVH, $q_2 = 3.40$, $p < .05$, rats ate more than controls, and the caloric intake of the two lesion groups were similar, $q_2 = 2.09$, $p > .05$. On high fat, both PVH, $q_2 = 5.63$, $p < .01$, and VMH, $q_3 = 7.55$, $p < .01$, ate more than controls; PVH and VMH rats consumed similar amounts, $q_2 = 1.92$, $p > .05$. On the second exposure to mash, the caloric intake of the three groups were similar, all pair-wise comparisons, were $p > .05$. When returned to high fat,

GROUP	N	DIET				
		Powder	Mash	High Fat	Mash	High Fat
Control	10	106.8 ± 2.0	121.2 ± 3.4	99.6 ± 3.0	102.6 ± 3.2	103.0 ± 3.5
PVH-lesion	7	126.4 ± 6.8	138.9 ± 6.8 ^a	123.3 ± 5.0 ^a	110.7 ± 3.7	125.9 ± 3.1 ^a
VMH-lesion	5	150.6 ± 14.9 ^{a,b}	149.8 ± 4.9 ^a	131.4 ± 4.6 ^a	101.8 ± 9.0	132.2 ± 11.3 ^a

Table 3. Mean (± SEM) daily food intake (Kcal/24hr) by PVH, VMH and control groups during each diet period. (a = significantly different from control at the .01 level; b = significantly different from PVH at the .05 level.)

however, both VMH, $q_3 = 5.20$, $p < .01$, and PVH, $q_2 = 4.08$, $p < .01$, groups were again hyperphagic.

Plasma Insulin and Glucose

The mean levels of plasma insulin and glucose are shown in Figure 4. Plasma glucose levels did not differ among the groups, $F(2,19) = 3.18$, $p > .05$. Plasma insulins, however, were different, $F(2,19) = 10.14$, $p < .01$. Multiple comparisons indicated that VMH rats were significantly hyperinsulinemic compared to both PVH, $q_2 = 5.17$, $p < .01$, and control, $q_3 = 6.30$, $p < .01$, rats. Although the mean insulin level of PVH rats was 55% higher than control values, this difference was not significant, $q_2 = 1.15$, $p > .05$. The differential effect of VMH and PVH lesions on plasma insulin is shown more clearly in Figure 5 which presents insulin values of individual animals. Only one PVH-lesion rat exhibited insulin levels beyond the control range. In contrast, 4 of 5 VMH rats demonstrated plasma insulin beyond the range of control values.

Body Compositions

Figure 4 and Table 4 illustrate the carcass analyses results. Analysis of variance indicated significant group differences in percent carcass fat, $F(2,19) = 40.19$, $p < .01$. Multiple comparison revealed that both the VMH, $q_3 = 11.98$, $p < .01$, and PVH, $q_2 = 7.72$; $p < .01$, groups were significantly fatter than controls. VMH rats were significantly fatter than PVH animals, $q_2 = 4.26$, $p < .01$. Further analysis showed significant group differences in amount of carcass

Figure 4. Group average physiological parameters of VMH.(N=5), PVH (N=7), and Control (N=10) rats maintained ad libitum. Rats were fasted for 24 hours prior to sacrifice. Vertical bars represent 1 SEM.

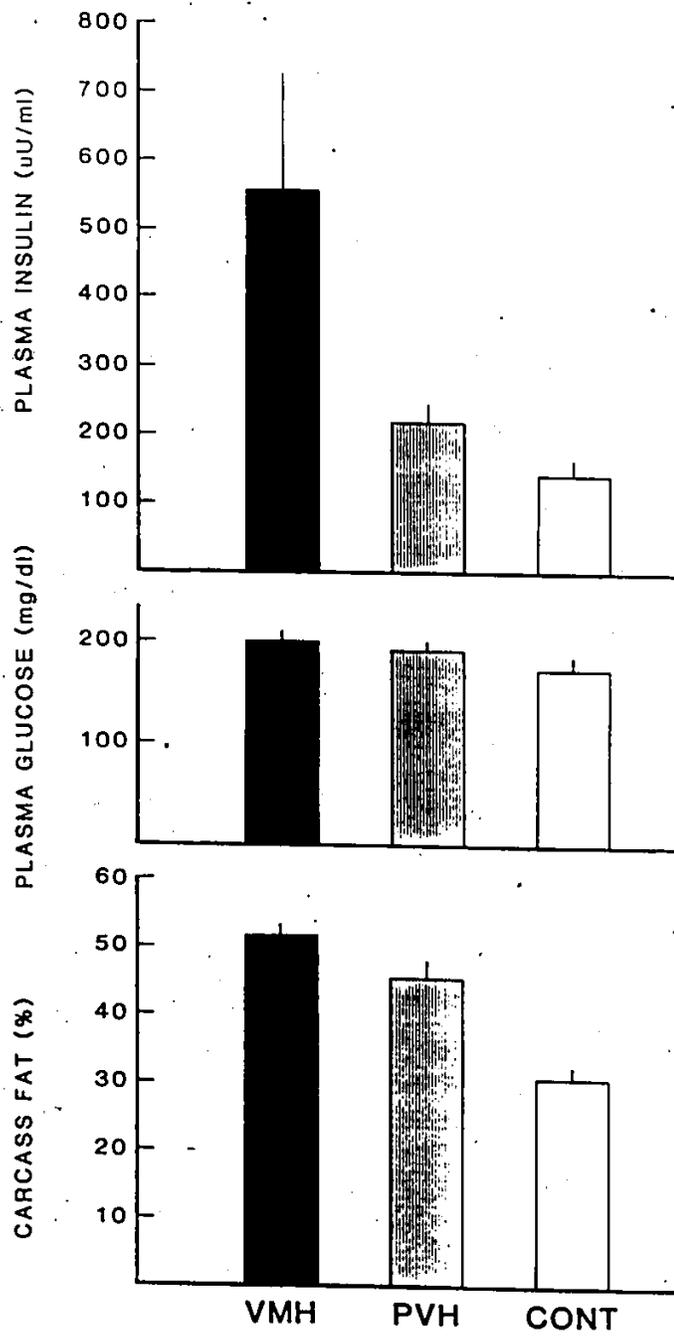
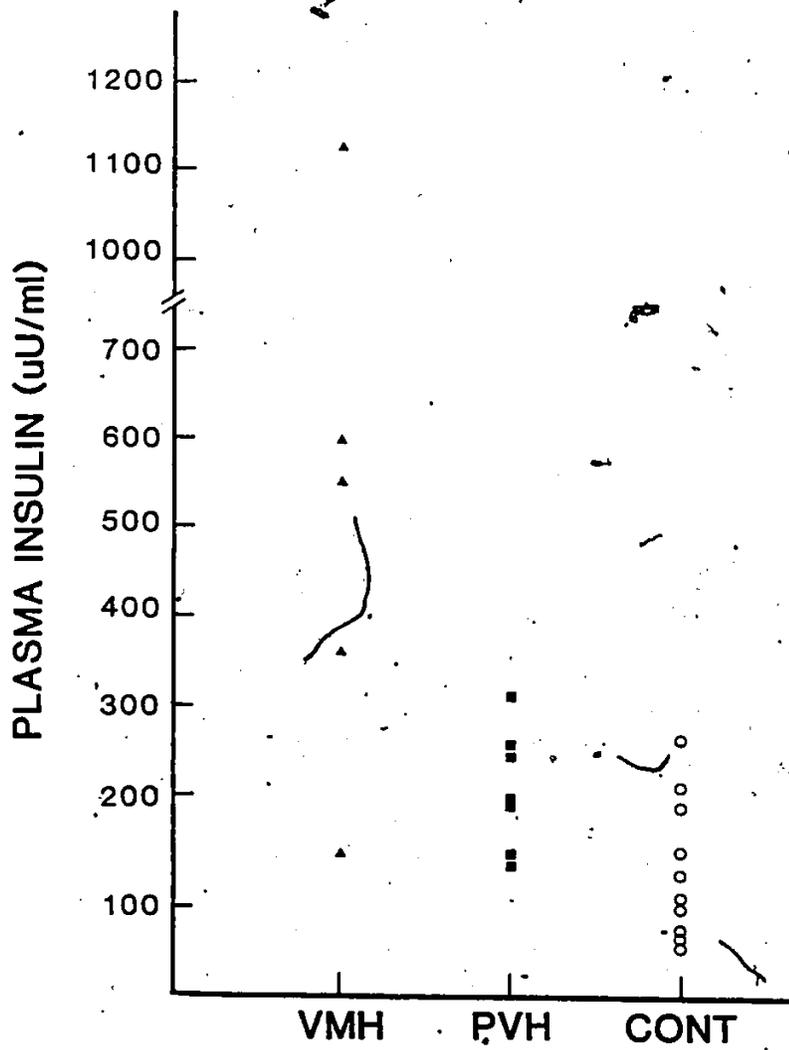


Figure 5. Individual levels of plasma insulin in VMH, PVH and sham lesion (CONT) rats maintained ad libitum.



<u>Parameter</u>	<u>Group</u>		
	<u>Control</u>	<u>PVH</u>	<u>VMH</u>
Carcass Weight (g)	510±13	700±19 ^a	745±29 ^a
Carcass Fat (g)	157±12	310±20 ^a	385±21 ^{a, b}
Carcass Water (g)	261±3	285±8 ^{a, c}	257±5

Table 4. Mean (± 1 SEM) physiological parameters of VMH, PVH, and Control groups. (a = significantly different from controls at .01 level. b = significantly different from PVH at the .01 level; c = significantly different from VMH group at .01 level.)

water as well, $F(2,19) = 6.71$, $p < .01$. PVH rats had greater carcass water than VMH, $q_3 = 4.81$, $p < .01$, and control, $q_2 = 4.12$, $p < .01$, rats. This body compartment was not different in VMH and control rats, $q_2 = .69$, $p > .05$.

Behavior Irritability

The irritability scores of the three groups are illustrated in Table 5. The nature of these data requires analysis with a nonparametric statistic. Thus, the Kruskal-Wallis analysis of variance by ranks procedure was used to assess group difference. Irritability scores of the groups were different, $H' = 11.47$, $p' < .05$. As indicated in Table 5, VMH rats were more irritable than control and PVH rats; no difference in irritability between PVH and control groups was apparent.

DISCUSSION

The results of this study indicate some similarities and some differences between the effects of VMH and PVH lesions. Both lesions induce hyperphagia, excessive weight gain and enhanced levels of body fat. However, the lesions differ in their ability to induce irritability and hyperinsulinemia. Specifically, unlike VMH rats, PVH animals do not demonstrate changes in affective behavior nor do they exhibit hyperinsulinemia.

The findings that PVH lesions induce hyperphagia and weight gain are consistent with previous reports (Aravich and Sciafani, 1983; Leibowitz et al., 1981; Sciafani and Aravich, 1983). The present data

<u>Irritability Test</u>	<u>VMH</u>	<u>Group</u>	
		<u>PVH</u>	<u>Control</u>
Response to pencil	.1	0	0
Visual presentation of hand	.4	0	.1
Biting response to hand	1.4	.2	0
Vocalization	1.4	.4	0

Table 5. Group mean irritability responses of PVH, VMH and control rats.

extend these findings in an important respect, however, by actually measuring carcass fat and showing that the weight gain of these lesion groups is a reflection of an increase in adipose tissue. However, under the present test conditions, hyperphagia and weight gain produced by PVH lesions differ in some respects from the VMH-induced disturbances. First, the hyperphagia and weight gain of PVH animals develop more slowly compared to VMH rats. This is apparent in the differences between the two lesion groups in the initial powder period. A slower development of hyperphagia in PVH rats is also alluded to by Sclafani and Aravich (1983). As well, on the second exposure to the mash diet, VMH rats lost weight whereas PVH animals did not. This may reflect an increased dependence of food intake on quality of diet in VMH rats compared to PVH animals. This is explored further in Chapter 5.

The present study demonstrates one clear behavioral difference between the two hypothalamic syndromes. Consistent with other reports (Grossman, 1972; Paxinos, 1973), the VMH animals in this study are irritable. PVH rats were not. Thus, it is apparent that PVH lesions do not damage the neural substrate producing this change in affect. This finding is reminiscent of an observation by Paxinos (1973) who showed that parasagittal knife cuts anterior to the VMN (i.e., in the area of the PVH) do not produce irritability. However, cuts lateral to the VMN do induce irritability. Thus, it appears that the neural substrate involved in producing changes in affective behavior is located posterior to the PVH and lateral to the VMN.

The metabolic profiles produced by the two hypothalamic lesions also differ in several respects. First, unlike VMH obesity, the PVH obesity is not associated with hyperinsulinemia. In the present experiment, the mean insulin levels of PVH rats were not significantly different from controls. Studies in the literature are equivocal on this issue. Sclafani and Aravich (1983) report that obese PVH rats show normal plasma insulin levels. In another study, however, they report a hyperinsulinemia in PVH rats (Sclafani and Aravich, 1982). They suggest that levels of plasma insulin in PVH animals may be modulated partially by carbohydrate content of the maintenance diet.

Body composition of the obese PVH rats is also somewhat different from the obese VMH rat. Unlike the VMH obesity, PVH lesions were not associated exclusively with changes in fat store. Consistent with previous findings (Cox and Powley, 1981; Han and Frohman, 1972), the body weight increase of VMH rats was associated only with an increase in body fat. In the obese PVH rat, however, not only body fat, but also body water were elevated. This indicates that the weight gain of the PVH rat is not due exclusively to changes in adipose tissue store.

CHAPTER IV. RELATIVE IMPORTANCE OF BEHAVIORAL AND METABOLIC CHANGES IN
THE DEVELOPMENT OF PVH- and VMH-INDUCED-OBESITY

An essential issue in investigating any obesity syndrome is identification of the relative importance of behavioral and metabolic changes to the development of the obesity. . Obesities are classified according to a behavioral or metabolic etiology (Mayer and Thomas, 1967). A behavioral, or "regulatory", obesity is one in which excessive fat levels characteristic of the obesity develop as a direct consequence of a behavioral disturbance; especially, a disturbance in the regulation of eating, i.e., hyperphagia. In contrast, a "metabolic" obesity is one in which some metabolic-dysfunction causes a change in body composition or physiology which leads directly to obesity. In this latter case, the development of obesity is independent of hyperphagia, although an excessive level of food intake can potentiate the degree of obesity.

The VMH lesion-induced obesity is metabolic in nature (Bray and York, 1979; Powley et al., 1980) as its development is independent of hyperphagia. In studies where the food intake of VMH rats is restricted to control levels, VMH rats still develop higher levels of body fat (Cox and Powley, 1981; Goldman et al., 1974; Han, 1967; Han and Liu, 1966). Similarly, metabolic disturbances following VMH lesions, such as hyperinsulinemia and gastric acid hypersecretion, exist even when food intake and body weight are normalized by restricted

feeding schedules (Cox and Powley, 1981; Han and Frohman, 1970; Han, Feng and Kuo, 1972; Hustvedt and Lovo, 1972; Karakash et al., 1977; Ridley and Brooks, 1965; Weingarten and Powley, 1980).

Although it has been suggested that the PVH obesity shares a similar etiology with the VMH syndrome (Scalfani and Aravich, 1983), support for this view is absent. First, no data exist to suggest that PVH lesion-induced obesity is hyperphagia-independent. As such, the possibility that PVH obesity is caused by excessive food intake cannot be precluded. Second, the effects of PVH lesions on metabolic profiles are unclear. Results indicating certain metabolic changes, e.g. hyperinsulinemia, after PVH lesion have not necessarily been replicated and, anyway, have been obtained in the already obese animal (Scalfani and Aravich, 1982; Steves and Lorden, 1982, 1984). As such, these data cannot be used to suggest a causal relationship between visceral hypersecretion and obesity since the observed metabolic changes may develop as a secondary consequence of hyperphagia (King et al., 1983, 1984).

This chapter consists of two experiments evaluating whether PVH and VMH obesities share similar etiology. The first experiment examines whether lesions of the PVH in fact induce a primary change in a visceral secretory event (gastric acid secretion). The second experiment evaluates the dependence of PVH obesity on postlesion hyperphagia. These experiments used a restricted feeding paradigm which normalizes the body weight levels of lesion animals to control values. This preparation is necessary to establish whether a certain result arises as a direct result of the neural damage per se or whether

it develops as a secondary manifestation of a hyperphagia or obesity. If the two hypothalamic lesion-induced obesities share a metabolic component, similar changes should be observed in PVH and VMH animals in the experiments to be described. In contrast, if the PVH obesity has a stronger behavioral component than VMH obesity, different results between the two lesion groups should be manifest.

EXPERIMENT 1. CHANGES IN GASTRIC ACID SECRETION FOLLOWING PVH AND VMH LESIONS

An elevation of basal gastric acid secretion is a defining index of the VMH syndrome. Basal hypersecretion occurs even when the hyperphagia of VMH rats is eliminated by restricted feeding (Ridley and Brooks, 1965; Weingarten and Powley, 1980). Since basal gastric acid levels are under direct vagal control, changes in level of acid secretion are interpreted as reflecting the degree of parasympathetic (i.e., vagal) tone on the visceral organs. It is not clear whether PVH lesions also produce a primary change in visceral secretion. In this experiment, changes in basal gastric acid secretion were measured following PVH or VMH lesions.

METHODS

Experimental Procedure

Subjects, at the beginning of this experiment, were 50 adult male Long-Evans hooded rats. Initially, all subjects underwent surgery for

gastric cannula implantation. Rats were allowed a 2-week recovery period following this surgery. During recovery, they were maintained ad libitum on Purina rat pellet.

The next 4 days of the experiment constituted a training period during which subjects became accustomed to the procedure and apparatus used to measure gastric acid.

Testing began after the training period. Rats were food deprived for 17 hours prior to a given test. Each test was 2 hours in duration, and the total amount of gastric acid secreted during that period was measured. Since acid secretion shows considerable inter-animal variability, this experiment was designed to permit an assessment of lesion-induced changes in acid secretion within each animal. Thus, a prelesion sampling period was included. Prelesion, acid secretion was monitored on 3 occasions; tests were conducted on alternate days. Then, subjects underwent stereotaxic surgery for PVH, VMH or sham lesions. All rats were food deprived overnight after surgery.

Over postlesion Days 2 to 12, acid secretion was monitored on alternate days yielding a total of 6 acid sampling tests. During this period, control rats had ad libitum access to Purina rat chow except prior to the acid sampling sessions when they were 17-hour food deprived. The daily food intake of lesion rats was restricted over this period so that the body weights of individual lesion rats remained equal to values of control rats matched for body weight at the time of stereotaxic surgery.

For 30 days following postlesion Day 12, all subjects were maintained ad libitum on Purina rat chow pellets. Body weights were recorded at the end of this ad libitum period.

The four days following the ad libitum period constituted a posttest during which rats underwent another two acid sampling trials. The mean level of acid secretion of each rat on these two tests was recorded as its posttest secretion level. At the end of this test, rats were sacrificed and brains removed for histological examination.

Measurements of Gastric Acid Secretion

Three different parameters of gastric secretion were obtained. First, the volume (expressed in mls) of the sample was measured. Second, the amount of acid (expressed as $\mu\text{Eq H}^+$) in the sample, i.e., acid output, was determined by titrating the sample to pH 7 with .05 M NaOH. Finally, the acid concentration (expressed as $\mu\text{Eq H}^+/\text{ml}$) of the sample was calculated by dividing acid output by sample volume. Due to the passive nature of the acid collection method, measurement of acid output might be confounded by the small amount of volume secreted in some cases. As such, it was generally assumed that the calculated measure of acid concentration provided the most sensitive index of stomach acid secretion.

Postlesion Acid Data

To take full advantage of the prelesion-postlesion design of this experiment, the effects of lesions on acid secretion were expressed by normalizing the postlesion acid data. Specifically, for each rat, the

mean prelesion acid data was assigned a value of 100%. Postlesion acid scores were expressed as a percentage of prelesion acid secretion (Normalized value = Postlesion/Prelesion X 100).

RESULTS

Histology

The histological criteria detailed in Chapter 3 were used to select animals into the PVH and VMH groups. Based upon these selection criteria, 10 PVH and 4 VMH rats were selected into the final lesion groups. Figure 6 illustrates the extent of 2 PVH and 2 VMH lesions.

Body Weight

The mean body weights of PVH, VMH and control groups are shown in Figure 7. The body weight of the three groups were equivalent at surgery, $F(2,21) = .15, p > .05$. Most importantly, the food restriction regimen successfully maintained the body weight of PVH and VMH rats at control levels during postlesion Day 2-12. During this period the body weight of the three groups were not significantly different, $F(2,21) = .004, p > .05$. However, the weight gained in the 4 weeks of ad libitum feeding were different, $F(2,21) = 6.34; p < .01$. Both PVH, $q_2 = 3.28, p < .05$, and VMH, $q_3 = 4.67; p < .01$, rats gained more weight than controls during this time; the weight gains of the two lesion groups were similar, $q_2 = 1.02; p > .05$.

Figure 6. Reconstruction of 2 PVH and 2 VMH lesions in the gastric acid study. Legend and abbreviations as in Figure 1.

7

	CONTROL RAT	PVH RAT	PVH RAT	VMH RAT	VMH RAT
FINAL BODY WT. (g)	443	584	559	601	568
NORMALIZED POSTTEST					
ACID CONCENTRATION (%)	135	187	116	204	162

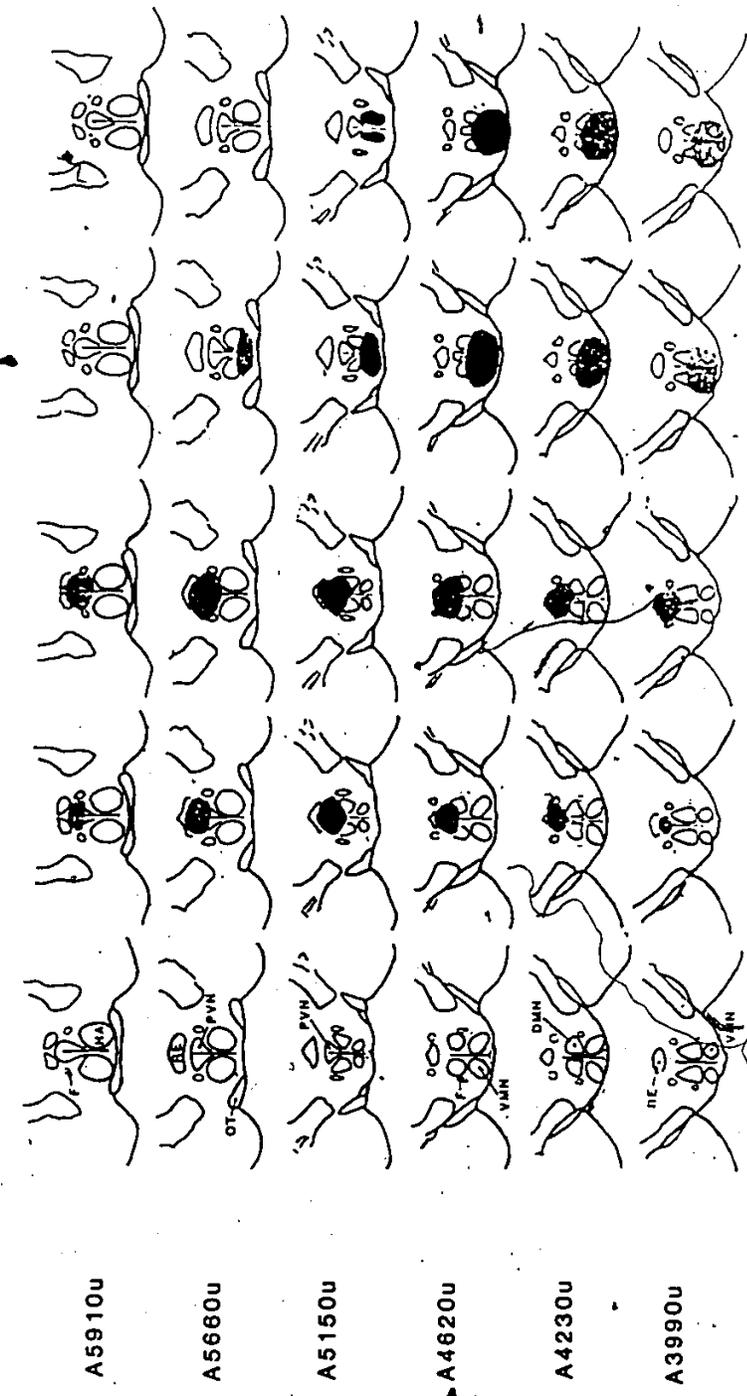
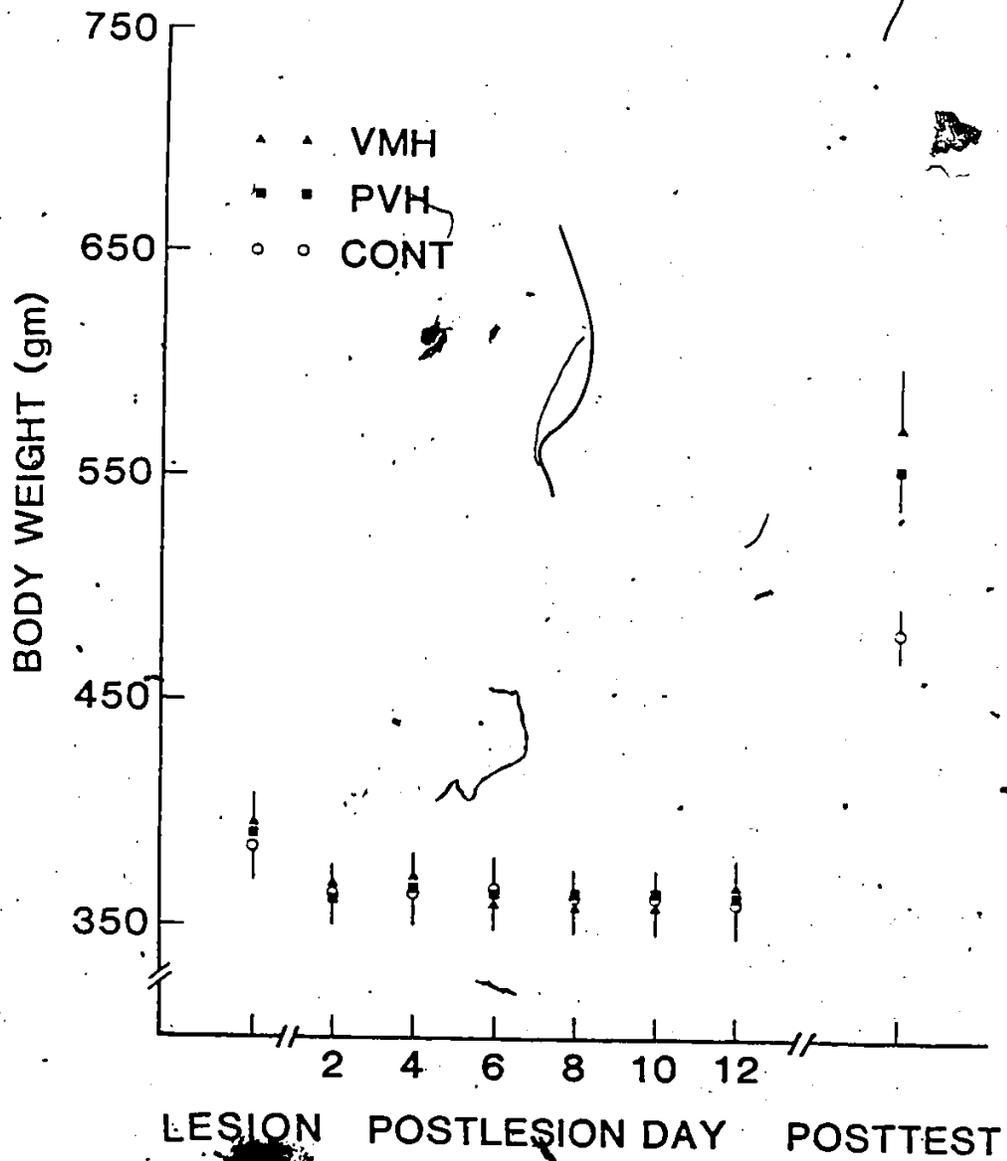


Figure 7. Mean body weight changes of VMH (N = 4), PVH (N = 10) and control (N = 10) rats in the gastric acid study. Vertical lines represent 1 SEM.



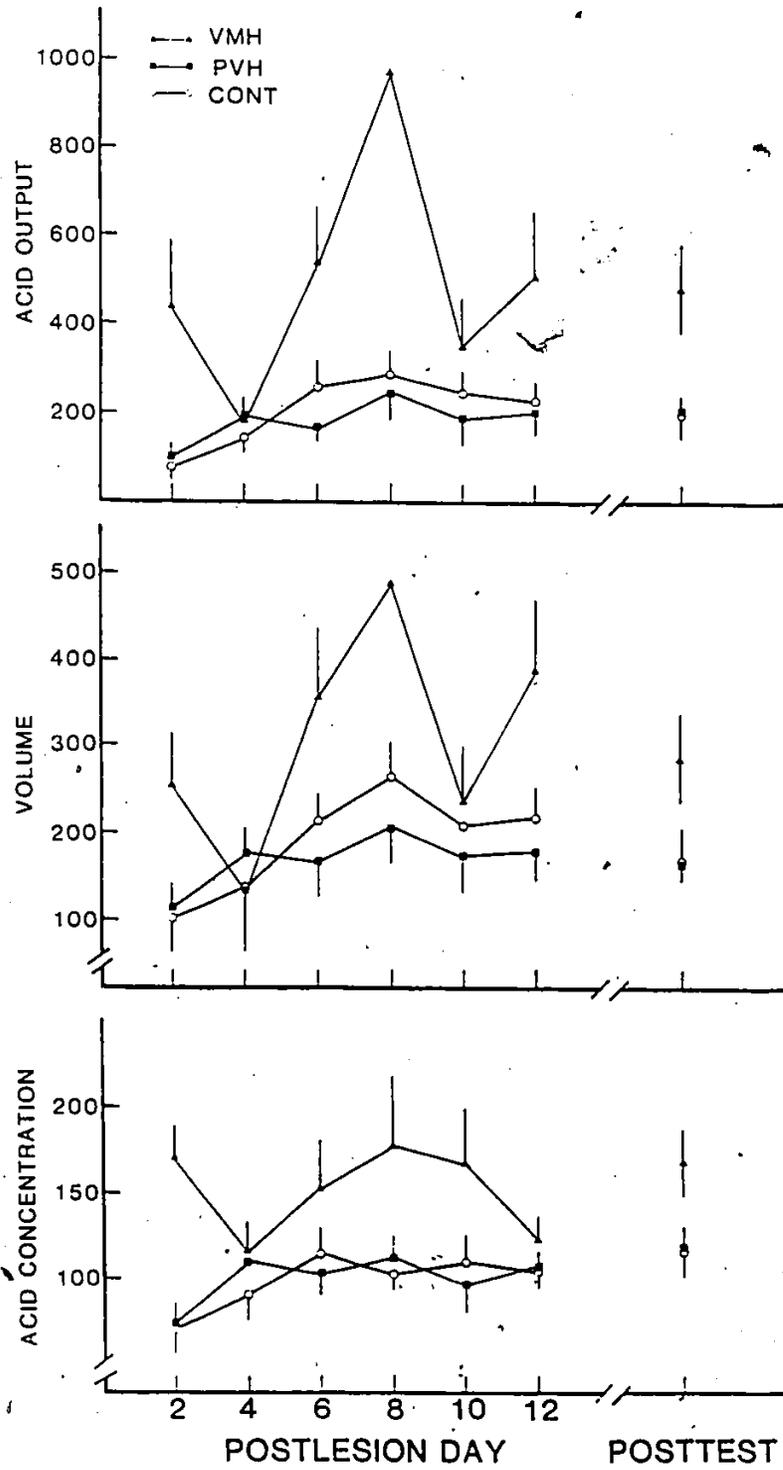
Gastric Acid Secretion

Prelesion, levels of gastric secretions were similar among the three groups in all three measures of secretion. Analyses of variance performed on the untransformed data indicated no group differences in acid output, $F(2,21) = 1.86$, $p > .05$; volume, $F(2,21) = 1.88$, $p > .05$; or acid concentration, $F(2,21) = 1.46$, $p > .05$. Analyses of normalized postlesion data indicated significant differences between the three groups in all these measures: acid output, $F(2,21) = 9.83$, $p < .01$; volume, $F(2,21) = 5.20$, $p < .01$; acid concentration, $F(2,21) = 7.09$, $p < .01$.

The postlesion gastric acid secretion of the three groups are showed in Figure 8. Gastric samples from VMH rats were significantly greater than controls in volume, $p < .05$, acid output, $p < .01$ and acid concentration, $p < .01$. VMH rats exhibited elevated secretions on Day 2 postlesion, p 's $< .01$ for volume, output, and concentration. On Day 4 postlesion, no group differences in any parameter were apparent, all p 's $> .05$. However, persistent elevation of acidity of gastric secretion was shown in all subsequent postlesion days, p 's $< .05$. Gastric secretion of PVH rats was similar to controls at all postlesion days, in terms of volume, output, and acid concentration.

Figure 8. Mean levels of basal gastric acid secretion in VMH (N = 4), PVH (N = 10) and control (N = 10) rats. Vertical lines represent 1 SEM.

NORMALIZED MEASUREMENTS OF GASTRIC ACID SECRETION (%)



DISCUSSION

The results of this experiment indicate that VMH, but not PVH, lesions elevate the basal level of gastric acid secretion. On every day postlesion, PVH rats had levels of gastric acid secretion nearly identical to control values. In contrast, VMH rats were hyperacidic in the postlesion period relative to both control and PVH rats. The hypersecretion was evident on Day 2 postlesion, although this may reflect some nonspecific effect of electrolytic lesions of the VMH such as tissue irritation (Schoenfield and Hamilton, 1977). Except on Day 4 postlesion, the hyperacidity of VMH rats persisted throughout the postlesion period. The exact cause for the failure to detect the hypersecretion on Day 4 is not clear.

The finding of a VMH lesion-induced basal hypersecretion is consistent with previous reports (Ridley and Brooks, 1965; Weingarten and Powley, 1980) and demonstrates several features of the lesion-induced change. First, gastric acid hypersecretion occurs in VMH rats even when hyperphagia is eliminated by restricted feeding. Second, gastric acid hypersecretion develops rapidly after VMH lesions; in the present test conditions, by Day 2 postlesion. This finding is in agreement with similar acid results (Ridley and Brooks, 1965) and consistent with data demonstrating rapid elevations of other visceral secretions following VMH lesions (Berthoud and Jeanrenaud, 1979; Rohner et al., 1977). Finally, additional body weight gain postlesion does not increase levels of acid secretion in VMH rats. As shown in the present study, the same levels of hypersecretion were manifest in VMH rats when

they were restricted to normal weights and when they became overweight after the ad libitum period.

This study reveals for the first time that lesions of the PVH do not alter basal levels of gastric acid secretion. Furthermore, hyperphagia and excessive weight gain of PVH animals which occurred during the ad libitum feeding period did not result in acid hypersecretion, even though PVH rats weighed more than controls at the time of this test. Although other studies have implicated the PVH in control of neuroendocrine or autonomically-regulated peripheral function (Ciriello and Calaresu, 1980; Rogers and Kahrilas, 1984; Taché et al., 1980), the absence of effects of PVH lesions on basal gastric acid or insulin (see Chapter 3) secretion suggest that the PVH is not a critical site involved in the central nervous system control of basal visceral secretion.

EXPERIMENT II. EFFECTS OF PVH AND VMH LESIONS ON BODY FAT IN RATS MAINTAINED ON RESTRICTED FEEDING

The previous experiment indicates that PVH and VMH lesions had different effects on visceral secretion. PVH rats exhibited normal levels of plasma insulin and gastric acid. In contrast, VMH animals were hyperinsulinemic and hyperacidic. In view of the relationship between these visceral secretions and the development of VMH obesity, these data suggest that the etiology of the PVH and VMH obesity may be different. This experiment provides the critical test of this

possibility by examining whether PVH obesity develops even in the face of normophagia. As discussed before, a "regulatory" obesity requires hyperphagia for the acquisition of excessive fat store. In contrast, in a "metabolic" obesity, excessive fat accumulates even with normal intake and body weight. VMH obesity is of this latter type. In this experiment, the classification of the PVH obesity is evaluated by examining body fat changes in PVH and VMH rats maintained at control weights by a restricted feeding procedure.

In this study, two different diets varying in carbohydrate content were used since it has been recently suggested that changes in metabolic profile after PVH lesions may depend on carbohydrate content of maintenance diet (Sclafani and Aravich, 1983). The diets used are Purina rat chow pellets and a high fat diet (see Table 1, page 27). Pellets have the higher carbohydrate content. If the development of PVH obesity depends solely upon increased consumption, i.e. a regulatory obesity, no changes in body fat composition should be found in PVH rats under either dietary conditions.

METHODS

Experimental Procedure

Seventy two male Long Evans hooded rats were subjects. They were weighed and assigned to six different groups so that the mean body weights would be equivalent. The groups differed in maintenance diet (Purina rat pellet versus high fat) and lesion type (VMH, PVH or sham ;

lesions). First, all rats underwent stereotaxic surgery. For the next 28 days, control rats had ad libitum access to their assigned diet, pellets or high fat. The food intake of the lesioned animals was restricted daily to ensure that their body weight gains matched that of their controls. At the end of the 28 days, subjects were sacrificed and carcass analyses conducted.

RESULTS

Histology

Eight PVH and 6 VMH rats maintained on high fat diet and 9 PVH and 6 VMH animals maintained on pellet diet were selected for the final lesion groups based upon the histological criteria described before. The extent of neural damage in 4 PVH and 4 VMH brains are illustrated in Figures 9 and 10.

Body Weight

Figure 11 illustrates the body weights of different groups throughout the study. The body weights of groups were similar at surgery, $F(5,39) = .14, p > .05$. Most importantly, the food restriction procedures used were successful in maintaining the weights of lesion animals at control levels under both dietary conditions. Although, overall, animals maintained on high fat gained more weight than those on pellets, $F(1,39) = 31.28, p < .01$, PVH and VMH rats gained similar amounts of weight as the appropriate control animals in both diet conditions.

Figure 9. Reconstruction of lesion damage in 2 PVH and 2 VMH rats maintained on pellets. Daily food intake was restricted to maintain weight at control levels.



	MEDIAN	FATTEST	MEDIAN	FATTEST	MEDIAN	FATTEST
	CONTROL RAT	PVH RAT	PVH RAT	PVH RAT	VMH RAT	VMH RAT
CARCASS FAT (%)	13.2	20.6	16.1	28.2	22.6	30
BODY WT. GAIN (g)	21	23	24	20		

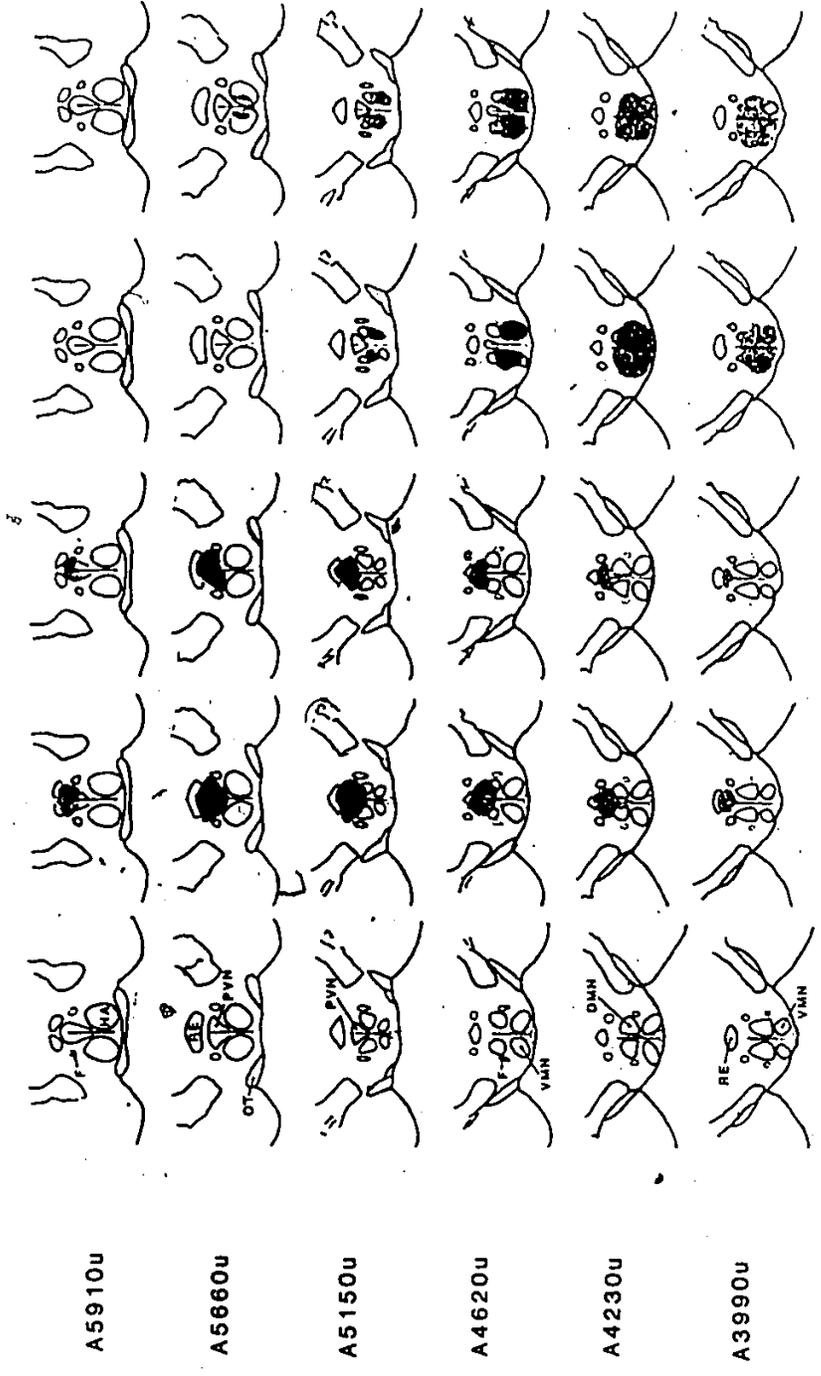


Figure 10. Reconstruction of lesion damage in 2 PVH and 2 VMH rats maintained on high fat. Daily food intake was restricted to maintain weight at control levels.

MEDIAN FATTEST MEDIAN

CONTROL RAT PVH RAT FATTTEST VMH RAT

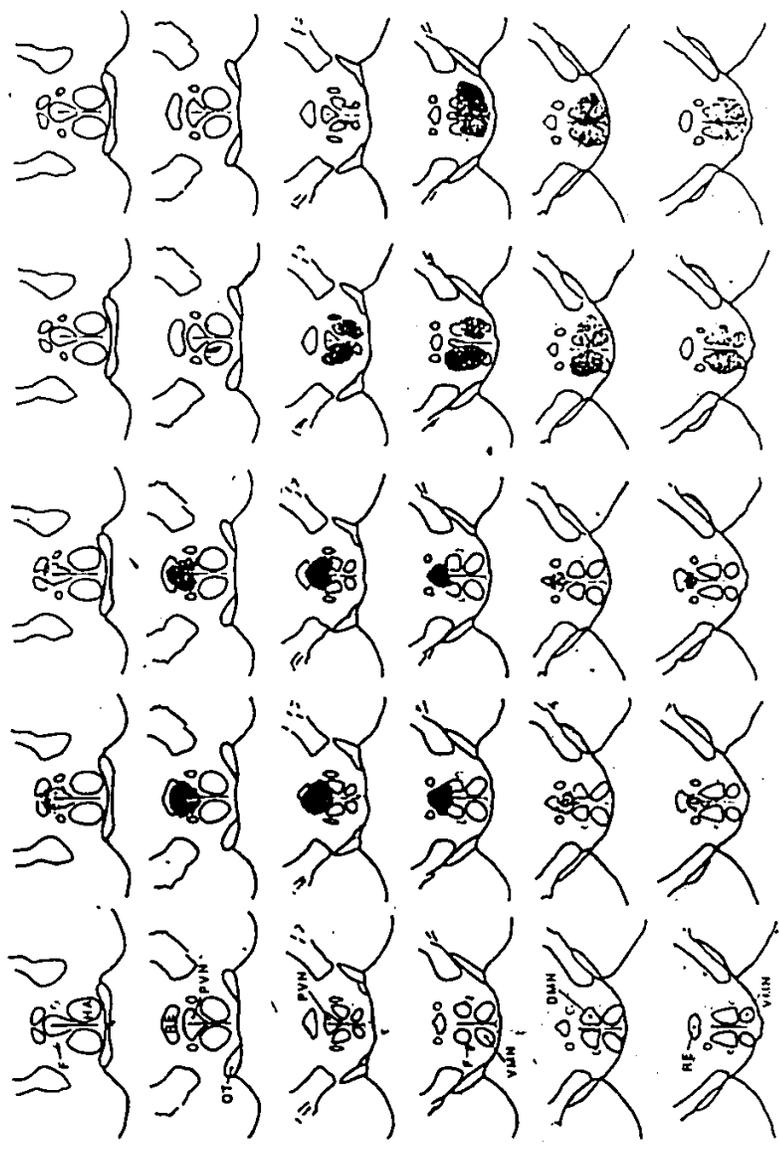
22.5 34.7 22.1 34.7 31.5

22.1 34.7 46

49 72 64 59

CARCASS FAT (%) 22.5 34.7 22.1 34.7 31.5

BODY WT. GAIN (g) 49 72 64 59



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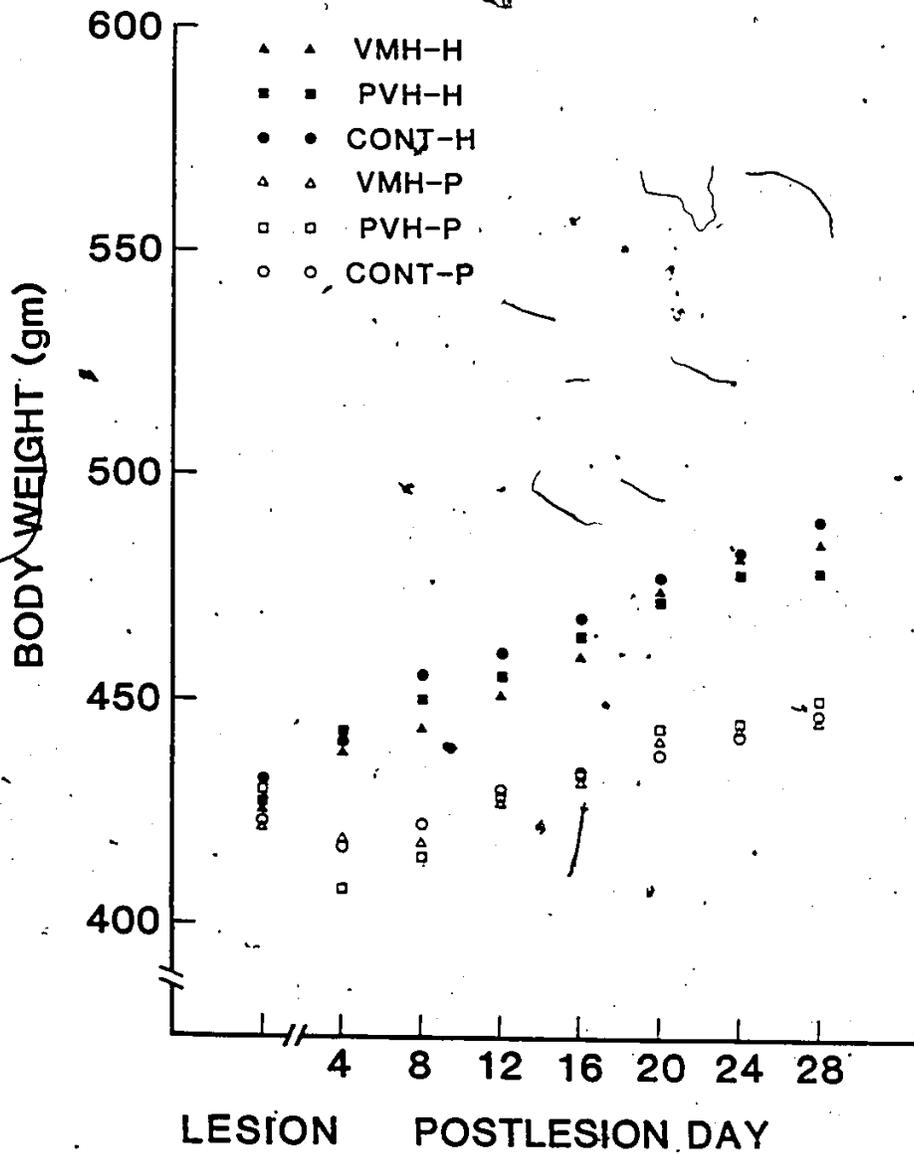
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Figure 11. Mean body weights of the groups throughout the 28 day postlesion period. Daily food intake of lesion groups was restricted to maintained their weights at control levels.



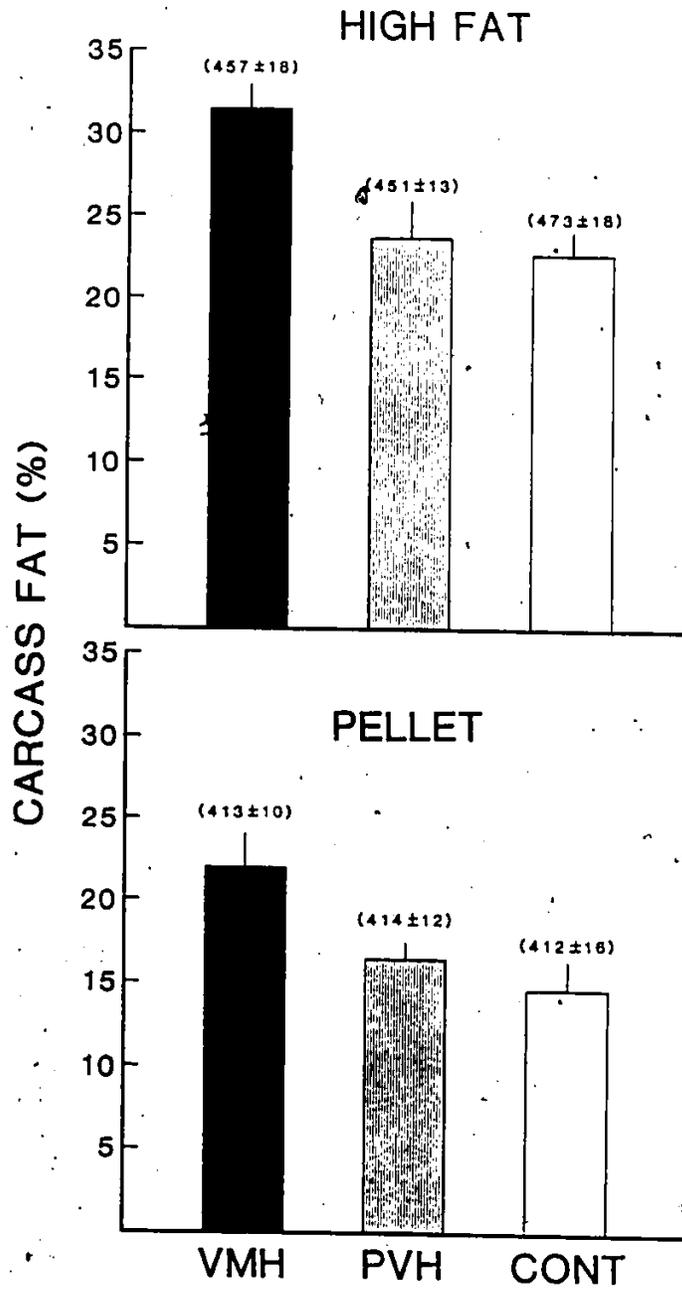
Carcass Compositions

The carcass analysis results are shown in Figure 12. Overall, animals maintained on the high fat diet were fatter than rats fed pellets, $F(1,39) = 37.86$, $p < .01$. Furthermore, group differences in percent fat were also apparent. VMH rats on pellets had a higher percentage carcass fat than control, $q3 = 4.42$, $p < .01$, and PVH, $q2 = 3.31$, $p < .05$, animals maintained on the same diet. Similarly, on high fat diet, VMH rats were significantly fatter than control, $q3 = 5.40$, $p < .01$, and PVH, $q2 = 4.79$, $p < .01$, animals. There were no significant differences in carcass fat between PVH and control animals in either the pellet, $q2 = 1.10$, $p > .05$, or high fat, $q2 = .61$, $p > .05$, conditions.

DISCUSSION

When the body weight increases of PVH and VMH rats are normalized by restricted feeding, VMH, but not PVH, rats develop an obesity. Specifically, greater percentages of body fat are found in normal-weight VMH rats maintained on either pellets or high fat. In contrast, PVH rats at control weights had nearly identical levels of body fat to control groups in both the pellet and high fat conditions. The present result of the development of obesity in food-restricted VMH rats is consistent with previous reports (Cox and Powley, 1981; Han, 1967). The findings in PVH rats, however, represent the first report of the failure of PVH rats to develop an obesity when maintained at control weight. The absence of obesity in normal-weight PVH rats indicates that the development of PVH obesity depends upon excessive food intake.

Figure 12. Mean percent carcass fat of the lesion and control groups maintained on high fat or pellet diets. Vertical bars represent ± 1 SEM. Numbers above bars indicate mean (± 1 SEM) body weight of group at sacrifice.



The present results also indicate that differences in composition of the diet do not interact with PVH lesions. PVH rats failed to demonstrate obesity on both a high carbohydrate and high fat diet. This finding argues against the hypothesis of Sclafani and Aravich, (1982; 1983) who postulate that the metabolic profile of the PVH rat depends upon the nutritional content of maintenance diet.

In concert with the results of the previous experiments, these data indicate that: i) PVH lesions do not produce a primary change in metabolic profile, and; ii) the development of obesity following PVH lesions depends critically on the opportunity for increased caloric intake. In contrast, acid hypersecretion and obesity develop in VMH rats even in the absence of hyperphagia. These findings suggest fundamentally different etiologies of the PVH and VMH obesities.

CHAPTER V. EFFECTS OF PVH AND VMH LESIONS ON SHAM FEEDING OF SUCROSE
SOLUTIONS VARYING IN PALATABILITY

The previous experiments demonstrate that PVH and VMH lesions have different effects on visceral secretion and fat deposition. This led to the suggestion that PVH obesity developed as a result of a primary disturbance in their control of feeding behavior. If this is so, then one should be able to detect changes in a feeding-related response even in PVH rats restricted to normal body weights. The present experiment provides this analysis by examining whether PVH rats also demonstrate the increased food intake in response to sensory properties of food, which is characteristic of VMH rats.

As discussed earlier, a defining trait of the VMH syndrome is a heightened reactivity to sensory qualities of food which manifests itself as exaggerated intake of good-tasting foods. It is not clear whether PVH lesions also produce a similar eating disturbance. PVH rats are more hyperphagic on certain foods than others (Aravich and Sclafani, 1983; Sclafani and Aravich, 1983). However, interpretation of these data is problematic since these data were obtained in obese PVH rats eating normally. For example, it is possible that the observed fluctuations in hyperphagia on different diets represents a secondary consequence of the developed obesity and not a direct result of the lesions. To assess this possibility it is necessary to test PVH rats prevented from becoming overweight. Furthermore, since the data

were obtained in normally feeding rats, they do not necessarily indicate that the feeding disturbance resulted from a change in reaction to sensory qualities of the foods. It may be that the lesion interacts with some postingestional consequence of the diets. A procedure is required to isolate food intake changes based solely upon sensory properties of the diet. Sham feeding is such a preparation. With sham feeding, the ingested diet stimulates the oropharynx but drains out of the stomach prior to the activation of gastric or intestinal mechanisms. Thus, this preparation eliminates the effects of postingestional or postabsorptive consequences on intake and thereby isolates the effects of oropharyngeal stimulation on food intake.

METHODS

Experimental Procedure

Thirty six male Long Evans hooded rats were used. First, a chronically-indwelling-gastric cannula was implanted into each rat. A two week recovery period was allowed during which time rats were maintained ad libitum on Purina rat pellets.

At the end of recovery, subjects were assigned to a VMH (N = 11), PVH (N = 16) or control (N = 9) groups. The mean body weights and variance of the three groups were equated. Rats underwent stereotaxic surgery for bilateral hypothalamic lesions or sham lesions. During the 7-10 day recovery period, control rats had free access to food. However, the daily food of PVH and VMH rats was restricted to maintain the weights of individual lesion animals equivalent to a control rat

equated for body weight at the time of stereotaxic surgery. This food restriction procedure continued for the duration of the experiment.

On 7 consecutive days following recovery from stereotaxic surgery, rats were habituated to the sham feeding environments and, more importantly, trained to lick reliably in the test cage. Training sessions were 30 min long and rats were permitted to sham feed 18% (weight/volume) sucrose solution. By the end of the training period, all subjects licked from the drinking spout immediately upon it being placed in the cage.

In the test phase, three test diets were used: 6% (weight/volume), 18% (weight/volume), and 36% (weight/volume) sucrose. On each test day, subjects were tested with only one liquid diet. The diet used on any particular day was selected randomly with the qualification that all test diets were represented within a 3 day block. This experiment was repeated four times producing a total of 12 test days. Each test session was 30 min in duration, and the amount ingested during consecutive 5 min of the session was recorded.

After testing, all rats were maintained ad libitum on a high fat diet for 2 weeks in order to measure the degree of hyperphagia and weight gain in PVH and VMH animals. The body weights of animals were recorded at the end of this period. Then, rats were sacrificed and the brains examined.

RESULTS

Histology

Seven PVH and 6 VMH rats were included in the final lesioned groups based upon the histological criteria established in Chapter 3. The extents of the lesions of 2 PVH and 2 VMH rats are shown in Figure 13.

Body Weight

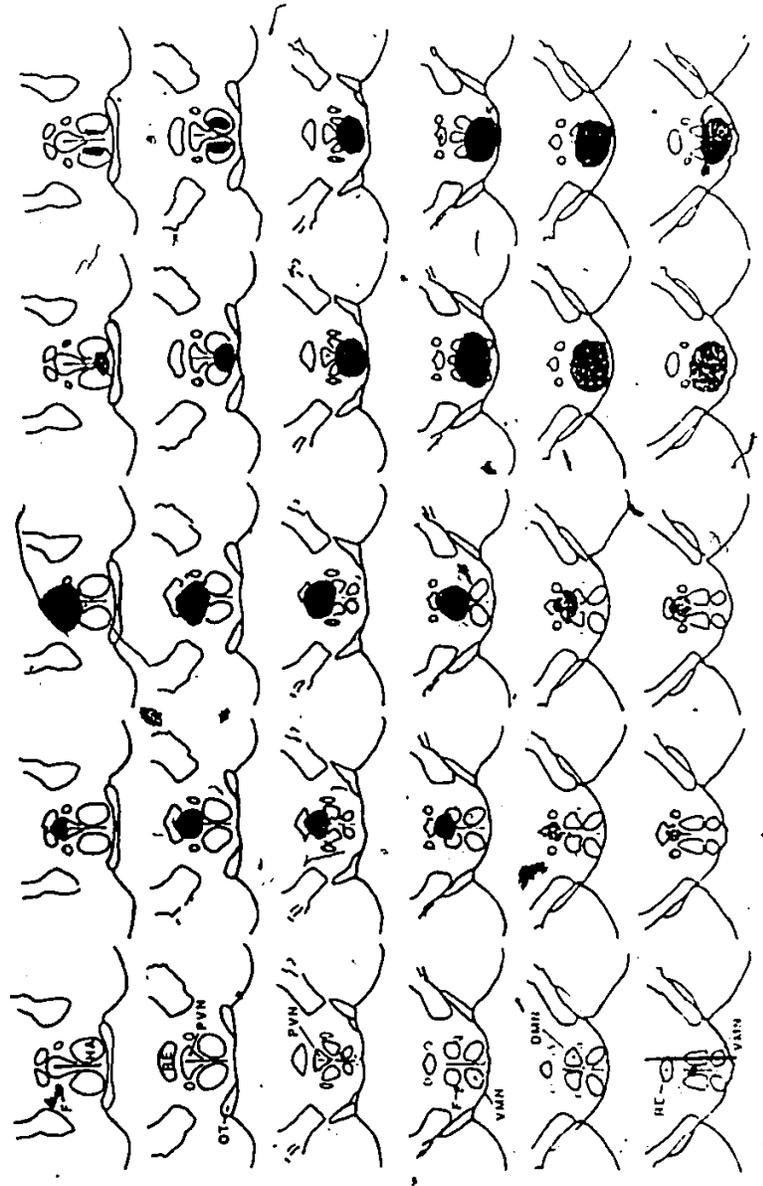
Figure 14 shows group mean body weights of the three groups at various phases of the experiment. The three groups had similar body weights at the time of stereotaxic surgery, $F(2,19) = .32, p > .05$. The food restriction procedure was successful in maintaining the body weights of lesioned animals at control levels throughout training and testing. Specifically, weights of PVH and VMH rats were not different from controls at the end of training, $F(2,19) = 2.8, p > .05$, or testing, $F(2,19) = 1.12, p > .05$. The body weight of the three groups were significantly different at the end of high fat, $F(2,19) = 12.92, p < .01$. Both PVH, $q_2 = 5.10, p < .01$, and VMH, $q_3 = 6.59, p < .01$, rats became significantly heavier than controls on high fat. Body weights of PVH and VMH rats were similar, $q_2 = 1.49, p > .05$.

Sham Feeding

The mean 30 min sham feed intakes of the three groups at the different concentrations of sucrose are shown in Figure 15. The amount sham fed by the groups was affected significantly by sucrose

Figure 13. Reconstruction of 2 PVH and 2 VMH lesions in the sham feeding study. Legend and abbreviations as in Figure 1.

	MEDIAN	LARGEST	MEDIAN	LARGEST	MEDIAN	LARGEST
	CONTROL RAT	PVH RAT	PVH RAT	PVH RAT	VMH RAT	VMH RAT
FINAL BODY WT. (gm)	468	665	540	691	640	640
30-Min	1.5P	7.5	2.5	6.75	3.75	3.75
SHAM FEEDING	ON 18%	ON 18.75	ON 20.5	ON 32.75	ON 30.75	ON 30.75
INTAKE (ml)	36%	39.5	35	38.25	35.5	35.5



A5910u

A5660u

A5150u

A4620u

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Figure 14. Group mean body weight of VMH (N=6), PVH (N=7) and control (N=9) rats during various phases of the sham feeding experiment. Vertical lines represent 1 SEM.

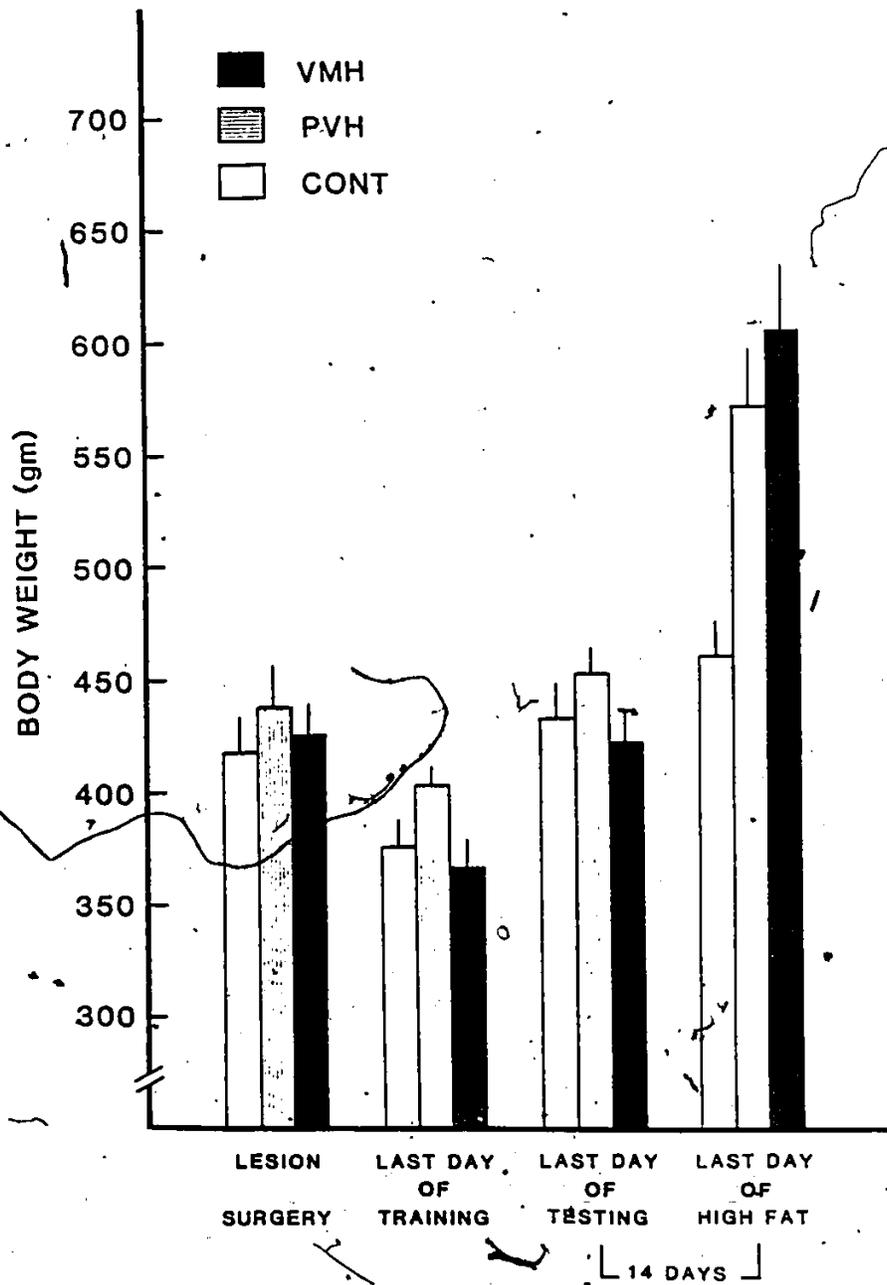
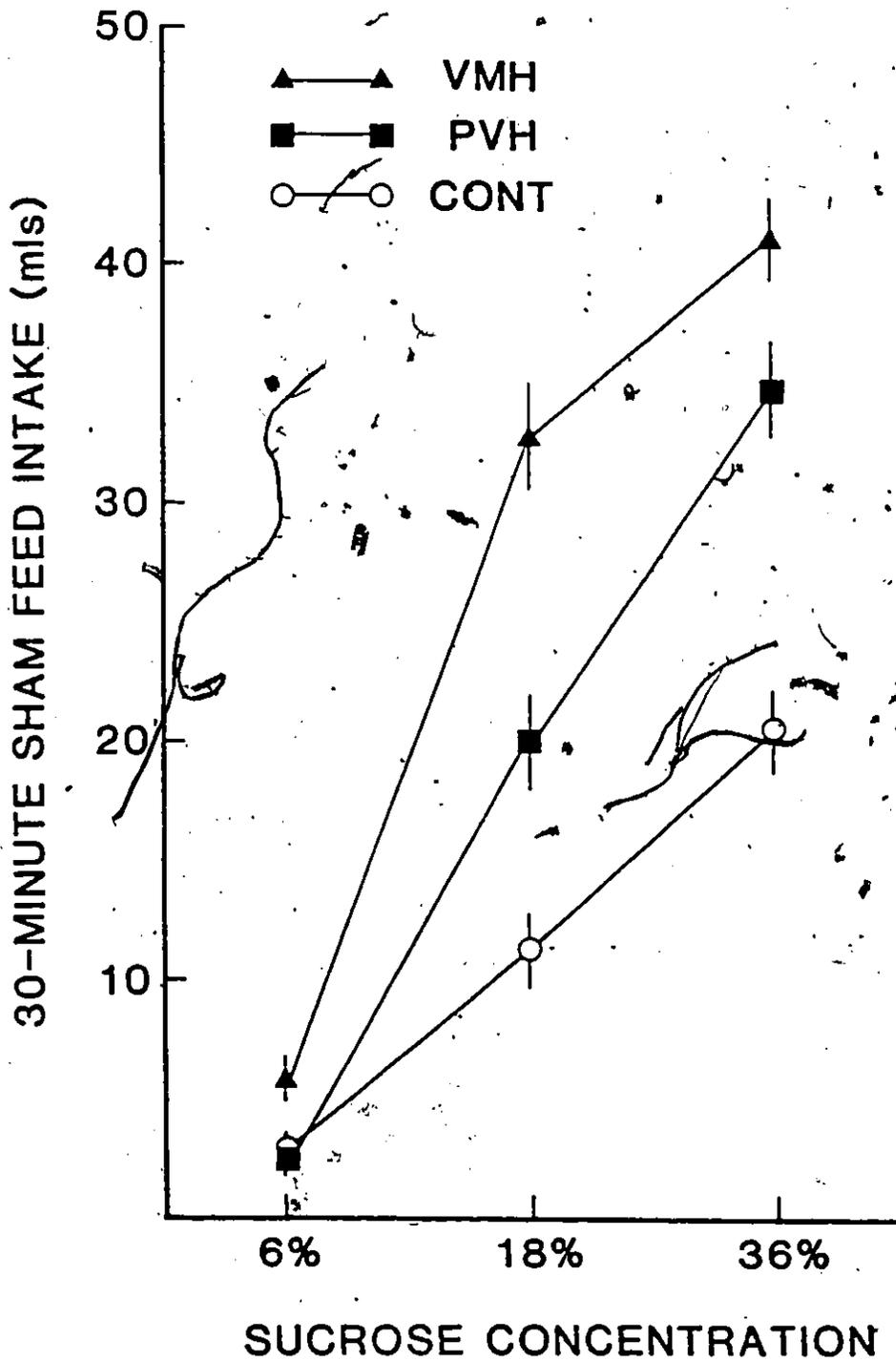


Figure 15. Mean 30-minute total sham fed intakes by VMH (N = 6), PVH (N = 7) and control (N = 9) rats of 6%, 18% and 36% sucrose. Vertical lines represent 1 SEM.



concentration, $F(2,38) = 262.98$, $p < .01$; all groups increased intake with ascending sucrose concentrations. But, the actual amount eaten by the three groups was also significantly different, $F(2,19) = 22.38$, $p < .01$. Further, the feeding profile of the three groups across the sucrose solutions differed significantly, $F(4,38) = 13.56$, $p < .01$.

Multiple comparisons were used to evaluate group differences at each sucrose concentration. The amounts ingested by the three groups were similar at 6% sucrose. On 6% sucrose, both PVH, $q_2 = .07$, $p > .05$, and VMH, $q_3 = 1.96$, $p > .05$, rats sham fed similar amounts as controls. At 18% sucrose, however, both PVH, $q_2 = 5.64$, $p < .01$, and VMH, $q_4 = 13.9$, $p < .01$, rats sham fed more than controls. PVH animals sham fed less than VMH rats, $q_3 = 8.26$, $p < .01$. Similar differences were apparent at 36% sucrose. PVH, $q_3 = 9.51$, $p < .01$, and VMH, $q_4 = 13.87$, $p < .01$, rats again consumed more than controls, and VMH rats drank more than PVH rats, $q_2 = 4.36$, $p < .01$.

DISCUSSION

The results indicate that both PVH and VMH animals exaggerate their level of food intake when the sensory properties of the diet are made more palatable. Under the present test conditions, higher concentrations of sucrose lead to increased sham feeding in control animals. However, this normal profile was amplified by PVH and VMH lesions. As the sucrose concentration of the diet increased, both PVH and VMH rats showed disproportionately large increases in the amount sham fed. The exaggeration of this response in PVH rats was attenuated

compared to VMH animals. As emphasized in the Introduction, sham feeding isolates the orosensory effects on food intake by eliminating the postingestive effects on consumption. Thus, these data suggest that both PVH and VMH rats are hyperresponsive to the sensory qualities of foods.

The present results in VMH and control animals agree with other sham feeding data (Weingarten, 1982; Weingarten and Wason, 1982) even though procedural differences (such as the degree of food restriction and level of body weight) exist between these studies.

The heightened responsiveness to taste qualities of food induced by PVH and VMH lesions did not represent a secondary consequence of postlesion body weight changes. In the present experiment, lesion animals were tested under normal body weights. Although it is possible that postlesion weight gain may further potentiate their responsiveness to the sensory aspects of food, it is clear from the present data that elevated body weight is not necessary for this behavioral disturbance.

CHAPTER VI. GENERAL DISCUSSION

The aim of this thesis was to characterize the PVH syndrome and, specifically, to compare the behavioral and metabolic disturbances accompanying PVH lesions to those produced by VMH damage. PVH and VMH lesions induce similar degrees of hyperphagia and weight gain under ad libitum feeding conditions, although the effects with PVH lesions are somewhat attenuated. The excessive food intake resulting from damage in the two hypothalamic sites results in the development of obesity identified as an increased body fat compartment relative to controls. In VMH animals, the obesity is accompanied by a hyperinsulinemia; insulin levels of obese PVH animals are elevated but are not significantly different from control values.

When the postlesion food intake of PVH and VMH animals is restricted to maintain their body weight at normal levels, VMH, but not PVH, animals develop an obesity. The development of obesity in VMH rats in the absence of hyperphagia is apparent regardless of whether the rats are maintained on a standard pellet, or a high fat, diet. In addition, only VMH rats at control or elevated body weights display a basal gastric acid hypersecretion. PVH rats do not demonstrate this disturbance regardless of weight level. Thus, the development of the obesity and specific visceral secretion changes following VMH lesions is hyperphagia independent. In contrast, the development of obesity following PVH lesions depends upon an excessive level of food intake.

Under sham feeding conditions, control rats increase consumption with ascending sucrose concentration. However, both PVH and VMH animals, even when restricted to control weights, show disproportionately large increments with increased sucrose, although this altered behavioral pattern is more apparent with VMH lesions. Thus, the data indicate that both PVH and VMH lesions produce the behavioral change of an altered reactivity to taste properties of food.

On the basis of these results, several conclusions can be made. First, the two hypothalamic lesions produce a similar set of feeding-related behavioral disturbances including hyperphagia, weight gain and an over-responsiveness to sensory properties of food. As such, it can be suggested that the PVH and VMH represent two sites controlling feeding behavior. Two possibilities exist. One is that these two sites represent points in a common hypothalamic circuit or system involved in the control of feeding. Alternatively, the feeding disturbances produced by lesions of these two loci may be mediated by independent mechanisms. This latter possibility is supported by the anecdotal observation that combined PVH and VMH lesions produced greater hyperphagia and weight gain than either PVH or VMH damage alone (Leibowitz, Hammer and Chang, 1981).

A second conclusion is that, unlike the VMH, PVH lesions do not produce a primary alteration of physiological and metabolic profile. In fact, failure to observe acid, insulin, or fat store changes in PVH lesion rats maintained at normal body weights suggests that the PVH is not critically involved in the control of secretion or metabolism.

The major conclusion from this project is that the pathogenesis of PVH and VMH lesion-induced obesities may be fundamentally different. As discussed in the General Introduction, obesities can be classified into two categories based upon the dependence of the obesity on an elevated food intake (Mayer and Thomas, 1967). The development of obesity in the presence of normophagia can be a defining test of a metabolic obesity. The requirement of hyperphagia for the development of an obesity may be used to define a regulatory obesity. The present results replicate the work of others (see Powley et al, 1980 for review) that the VMH syndrome represents a metabolic obesity in that the development of the obesity does not depend upon an excessive level of food intake. Most other animal models of obesity including the Zucker rat (Cleary, Vasselli and Greenwood, 1980), ob/ob mouse (Chlouverakis, 1970) and db/db mouse (Cox and Powley, 1977) are examples of metabolic obesities. Hyperphagia amplifies the anabolic disturbances in these metabolic obesities. In contrast, the PVH obesity appears to be regulatory in nature as its development depends critically on the opportunity for overeating.

It is unlikely that differences documented between PVH and VMH syndrome exist simply because of differences in the amount of hypothalamic tissue destroyed by the two lesions. First, although less current is used to produce PVH lesions (relative to VMH lesions), the PVH lesions are far from small. The typical PVH animal in these experiments sustained extensive hypothalamic damage in the rostral dorsal hypothalamus including complete destruction of the paraventricular nuclei. Furthermore, animals with VMH lesions smaller

in size than the typical PVH damage sustained by animals selected for the PVH groups in these studies exhibit elevated visceral secretion and become obese (Bernardis and Frohman, 1970, 1971). Second if simply the amount of tissue damage in the hypothalamus (regardless of locus) contributed to the magnitude of these physiological changes, one would expect to observe some degree of disturbance in PVH animals. However, as demonstrated in the present and other experiments (Gunion and Taché, 1985), levels of gastric acid secretion and body fat are almost identical in control and PVH animals restricted to normal weights.

Recently, the PVH has been identified as a hypothalamic site with intimate connections with the central autonomic nuclei such as the dorsal motor nucleus of the vagus. On the basis of these anatomical connections, some have argued for a role of the PVH in the regulation of peripheral metabolism (Sawchenko and Swanson, 1981; Swanson and Kuyper, 1980; Swanson and Sawchenko, 1980). Other studies implicate the PVH in the control of peripheral function. For example, electrical stimulation of the PVH increases gastric acid secretion (Rogers and Kahriles, 1984). However, the complete inability of PVH lesions in the present studies to produce changes in basal gastric acid secretion or fat storage suggest that the control of these peripheral events by the PVH is not simple.

These conclusions have implications for the analysis and interpretation of the collection of disturbances comprising the hypothalamic lesion syndromes. One traditional approach has been to suggest that the hypothalamic ablation induces a single primary disturbance which leads, secondarily, to the development of other

characteristics comprising the syndrome. In the case of the VMH syndrome, the disturbance identified as the primary causal factor has changed over time. Originally, hyperphagia was considered as the primary causal factor (Brobeck, Tepperman and Long, 1943). More recently, the primacy of metabolic disturbances, such as elevated visceral secretions (Bray and York, 1979; Powley, 1977), has been championed. There is however, an alternative interpretation of the collection of physiological and behavioral disturbances following hypothalamic lesions. The hypothalamus is a complex, heterogenous structure involved in many aspects of homeostasis and behavior. It is possible that large VMH lesions induce a series of independent disturbances which, given the appropriate anatomical and behavioral analyses, could be dissociated from one another. Although this sentiment has been expressed (Grossman, 1984; Powley et al., 1980; Sclafani and Kirchgessner, 1985), this approach is not usually represented in experimental analysis of the hypothalamic obesity syndromes. As discussed earlier, the results of the present series of experiments indicate that lesions of two adjacent areas of the hypothalamus produce obesities with fundamentally different etiologies. Others have also argued for a distinction between a VMH syndrome induced by electrolytic lesions and by parasagittal knife-cuts (Bray, Sclafani and Novin, 1982). In contrast to the VMH lesion syndrome, neither the knife-cut nor the PVH animal is hyperinsulinemic. The possibility exists, therefore, the two types of obesity can be produced by damage of the hypothalamus. One, represented by the VMH knife cut and PVH lesion, produces a primary disturbance in food intake. The second,

characterized by VMH electrolytic lesions, produces a primary set of metabolic disturbances which result in increased adiposity. Since lesions restricted to the ventromedial hypothalamic nuclei are sufficient to produce a metabolic obesity (Bernardis and Frohman, 1970, 1971), but not hyperphagia (Beven, 1973; Gold; 1973) it is possible that larger lesions of the VMH, such as those used in the present as well other experiments to produce VMH syndrome, might destroy both of these systems and, therefore, produce a more pronounced obesity.

One fundamental question remains: why do PVH and VMH rats overeat? There is no generally accepted answer although several possibilities can be entertained. One possibility is that the hyperphagia may result from a insensitivity to satiating signals which arise normally from the periphery and signal meal termination. Ingested food elicits a variety of physiological satiety signals (e.g., gastric distention, release satiety hormones, etc.). If hyperphagia-inducing hypothalamic lesions disrupt processing of these satiety signals, overeating may result. Experimental results, however, indicate that such conjecture may not be true. Several studies demonstrate that hyperphagic VMH animals are equally responsive as normals to "satiety" manipulations such as intragastric preloads (Liu and Yin, 1974; McHugh et al., 1975; Novin et al., 1979; Panksepp, 1971; Smith et al., 1961; Thomas and Mayer, 1968) and injection of putative satiety hormones, such as cholecystokinin and bombesin (Kulkosky et al., 1976; West et al., 1982). Thus, these data mitigate the interpretation of hyperphagia resulting from a satiety mechanism deficit.

An alternative explanation for hypothalamic lesion-induced hyperphagia is that the overeating may result from an overresponsiveness to mechanisms which normally stimulate eating. PVH and VMH rats demonstrate exaggerated food intake in response to the taste of food. It is possible that a disturbance related to this deficit of sensory processing contributes to the overeating.

In summary, the present experiments demonstrate similar effects of PVH and VMH lesions on producing feeding related behavioral changes and reveal different effects of the two lesions in producing metabolic disturbances. These results suggest that the etiologies of the two obesity syndromes are fundamentally different. PVH lesions induce a regulatory obesity, VMH lesions induce a metabolic obesity. In addition, these results implicate that the elements of hypothalamic obesity syndromes may be independently induced by damage to separate hypothalamic systems which control different aspects of feeding and energy metabolism.

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