# A DISSOCIATIVE ANALYSIS OF THE VMH SYNDROME

# DISSOCIATION OF THE BEHAVIOURAL AND METABOLIC DISTURBANCES IN THE VENTROMEDIAL HYPOTHALAMIC OBESITY SYNDROME

BY

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## ABSTRACT

Electrolytic lesions of the ventromedial hypothalamus produce an obesity syndrome in experimental animals characterized by behavioural and metabolic disturbances. Historically, theories of VMH obesity have considered a single disturbance, either behavioural or metabolic, to be the primary effect of the lesion, which in turn causes other components of the syndrome. An alternative view suggests that VMH lesions simultaneously disturb both behavioural and metabolic mechanisms due to the anatomical proximity of these mechanisms in the hypothalamus. Therefore, more discrete lesions in the VMH may produce some syndrome components but not others. This thesis presents a series of experiments that test this "dissociative" perspective of the VMH obesity syndrome.

First, rats having different hypothalamic ablations were compared on: caloric intakes on a series of test diets, body weight changes, and body fat. Bilateral parafornical hypothalamic knife cuts (PFKC) that spared the ventromedial hypothalamic nucleus (VMN), produced overeating and weight gain characteristic of VMH lesions. However, measurement of percentage body fat (i.e. level of obesity) indicated that PFKC rats were less obese than VMH rats, even though PFKC lesions produced a greater hyperphagia and weight gain than VMH lesions. In contrast, lesions restricted to VMN

produced obesity, but did not produce hyperphagia or weight gain.

Since parafornical knife cuts produced a greater hyperphagia than VMH lesions, it is possible that VMN damage actually reduces caloric intake in VMH rats. To test this hypothesis, the effects of VMH, PFKC, and combined PFKC/VMN lesions on caloric intake and body weight were compared. PFKC and VMH lesions produced hyperphagia and weight gain. However, knife cuts were not significantly more effective than VMH lesions for producing these disturbances in this experiment. Therefore, PFKC lesions do not invariably produce a greater hyperphagia than VMH lesions. Furthermore, VMN lesions had no effect on the level of overeating or weight gain in rats bearing PFKC lesions. Therefore, damage to VMN does not reduce the hyperphagia produced by PFKC lesions.

Finally, the effects of these different hypothalamic manipulations on metabolic measures were determined. To eliminate the confound of hyperphagia on metabolic variables, all lesion rats were fed a daily food ration sufficient to maintain their body weight at the level of controls. VMH and PFKC lesions resulted in elevated parasympathetic tone, indicated by elevated basal gastric acid secretion. VMN lesions did not affect gastric acid secretion. In contrast, only VMH and VMN lesions produced obesity when overeating was prevented. PFKC rats did not become obese.

These experiments demonstrate that separate hypothalamic mechanisms underly the hyperphagia and obesity characteristic of VMH lesions. Furthermore, different mechanisms underly obesity and elevated parasympathetic tone following VMH lesions. Therefore, these observations support a dissociative model of the VMH obesity syndrome.

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# CHAPTER 1. INTRODUCTION

## I. THE VMH SYNDROME

An experimental obesity is produced in rats by making lesions bilaterally in the area of the brain termed the ventromedial hypothalamus (VMH). VMH obesity has served traditionally as a model for studying the behavioural and physiological determinants of eating, body weight and adiposity. The attractiveness of the VMH animal obesity model is due largely to important practical and theoretical advantages provided by this preparation. First, a profound and rapid obesity is produced by relatively simple experimental techniques (i.e. lesions of the VMH). Second, the phenomenon shares many of the behavioural (Schachter, 1971) and metabolic (Bray, 1976; Rohner-Jeanrenaud, Bobbion, Ionescu, Sauta, & Jeanrenaud, 1983) characteristics of naturally-occurring obesities. Finally, the phenomenon identifies the ventromedial hypothalamus as a brain site that is important in the modulation of energy stores, possibly through metabolic or behavioural mechanisms.

The rat was used initially to model the obesity associated with basomedial hypothalamic lesions observed in humans (Mohr, 1840, cited in Broebeck, 1946; Frohlich, 1901). However, since the original demonstrations of VMH lesion-induced obesity in the rat (Hetherington & Ranson, 1939; Broebeck, Tepperman, & Long, 1943), an experimental obesity has been produced by VMH lesions in a variety of other animals including:

mouse (Mayer, French, Zighera, & Barrnett, 1955), golden hamster (Marks & Miller, 1972), rabbit (Balinska, 1963; Romaniuk, 1962), ground squirrel (Mrosovsky, 1974, 1975), dog (Roskowska & Fonberg, 1971), rhesus monkey (Hamilton & Brobeck, 1964), chicken (Lepkovsky & Yasuda, 1966), goose (Auffray & Blum, 1970; Snapir, Yaakobi, Robinzon, Ravona, & Perek 1976), white-throated sparrow (Kuenzel & Helms, 1967) and swine (Auffray, 1969: Khalaf, 1969).

A variety of behavioural and metabolic effects of VMH lesions have been documented and, therefore, the phenomenon is now referred to as an obesity 'syndrome'. However, most of the research on the syndrome has been conducted on a subset of disturbances that are believed to be relevant to the obesity. The following disturbances are commonly considered defining components of the VMH syndrome.

## Defining Components of the VMH Obesity Syndrome

### 1. Hyperphagia

The most studied behavioural effect of VMH lesions is overeating. Rats are hyperphagic immediately upon awakening from the anesthetic used in the lesion surgery (Broebeck, et al., 1943). The degree of hyperphagia produced by electrolytic VMH lesions changes with time post-lesion such that three distinct phases can be identified. These have been labelled the "acute dynamic", "dynamic", and "static" phases.

The "acute dynamic phase" lasts only a few hours and is marked by frenzied eating behaviour (Balagura & Devenport, 1970). Overeating at this stage may be better labelled polyphagia or bulimia than hyperphagia (Powley, Opsahl, Cox, & Weingarten, 1980) as it appears to occur in the

absence of control by normal orosensory and gastrointestinal mechanisms. During this period, eating can continue until the animal bloats (Harrell & Remley, 1973) and there is a risk of asphyxiation (Broebeck, et al., 1943). If food is unavailable during this period, the animal may consume large quantities of almost any ingestible non-nutritive material. To prevent asphyxiation during the acute dynamic phase, VMH lesion animals are routinely food deprived for approximately 24 hours after lesions. Following the acute dynamic phase, postingestive and sensory control over eating return and sensory discrimination is even exaggerated, as discussed in more detail below (see "finickiness").

The "dynamic phase" of hyperphagia, which is often considered to start immediately post-lesion, is a period of dramatic overeating and weight gain. The caloric intake of VMH rats during this period can be 2 to 3 times their normal daily intake (Corbit & Stellar, 1964). Weight gain can occur at a rate of about 5% of prelesion weight per day (Gold, 1970). The duration of the dynamic phase is a function of lesion size (Reynolds, 1959). In the majority of experiments, relatively large lesions are employed and, therefore, the dynamic phase persists for a month or longer.

The "dynamic phase" eventually gives way to the "static phase" when the rate of body weight gain reduces or stabilizes and levels of caloric intake return to near normal (Brooks & Lambert, 1946). Although the degree of hyperphagia diminishes with time, it is also clear that the static phase is not a recovery of control over food intake, because if a static phase animal is food restricted and loses weight, it will re-enter the dynamic phase and exhibit hyperphagia when food is returned ad libitum (Brooks & Lambert, 1946; Hoebel & Teitelbaum, 1966).

Hyperphagia is characteristic not only of VMH rats but also of humans with lesions in the area of the ventromedial hypothalamus. Bray and Gallagher (1975) measured levels of caloric intake in a female patient who suffered hypothalamic injury as a secondary consequence of surgical intervention. The patient was described as having "uncontrollable hyperphagia". However, the reported caloric intake of this patient would appear to be a relatively modest level of overeating. Her intake over a 37 day period ranged between 400 and 3400 kilocalories per day with a mean of 2100 kilocalories (Kcal). This level exceeded metabolic requirements by 400 Kcal and led to a weight gain of eight pounds. Other reports have indicated more striking hyperphagia. One four-year-old girl with inflammatory hypothalamic lesions was found to consume 4500-6000 Kcal per day (Heldenberg, Tamir, Ashner, & Werber, 1972). An adult patient with VMH neoplasm was reported to consume 8,000 - 10,000 Kcal per day (Reeves & Plum, 1969). Whether the dynamic and static phases of hyperphagia occur in human VMH obesity is not clear. Typically, lesions in humans are produced progressively by disease processes and, therefore, such an analysis is not possible.

2. Finickiness

The caloric intake and weight gain of VMH rats is highly dependant on the diet upon which animals are maintained. More palatable diets promote a proportionately greater hyperphagia and obesity and, therefore, the VMH animal has been described as 'finicky' (Teitelbaum, 1955). Finickiness is defined as an inordinate sensitivity to the sensory aspects of food (Powley, 1977). In a series of experiments by Teitelbaum (1955) it was demonstrated that a greater hyperphagia and weight gain, in VMH animals

relative to controls, could be achieved by varying the texture and taste of the diet. Chow given in pelleted form supported a greater caloric intake than the same diet provided in powdered form. Obese VMH rats were also more hyperphagic when 50% dextrose (sweet tasting) was added to their regular chow diet, whereas the addition of dextrose had no effect on the caloric intake of controls. Moreover, adding 0.125% quinine (bitter tasting) to the same diet produced a marked reduction in consumption, but had only a slight effect on controls. Thus, the 'finickiness' of VMH rats appeared to be bidirectional. This negative finickiness was impressive because excessive weight gain by VMH lesions could be completely prevented by simply feeding rats unpalatable food (see also, Ferguson & Keesey, 1975).

Finickiness is interpreted, most commonly, as reflecting a primary sensory disturbance by VMH lesions. An alternative interpretation is that finickiness reflects a preference by VMH rats for foods that are higher in caloric value. However, inordinate levels of overeating can be produced by simply changing the texture of food (Corbit & Stellar, 1964; Kramer & Gold, 1980; Sclafani, Aravich, & Schwartz, 1979; Teitelbaum, 1955), or by adulteration with non-nutritive oil (Carlisle & Stellar, 1962). Even providing VMH rats with fresh food more often can produce a greater hyperphagia (Broebeck, 1946). Finally, rats with unilateral VMH lesions, which do not produce hyperphagia, show a preference for more palatable food when it is placed in the sensory field contralateral to the lesion (Marshall, 1975).

The most effective manipulation of palatability for affecting VMH hyperphagia and weight gain is to increase the fat content of the diet (Corbit & Stellar, 1964; Lundback & Stevenson, 1947; Teitelbaum, 1955).

Although "sweetness" might be predicted to be important, foods high in simple carbohydrate produce, at best, only a modest hyperphagia relative to lab chow (Teitelbaum, 1955; Graff & Stellar, 1962). Furthermore, when rats are provided with a choice of diets rich in either fat, carbohydrate, or protein, VMH lesions shift selection of macronutrients towards a greater consumption of fat as a percentage of total daily calories, with reduced percent carbohydrate and protein intake (Kanarek, Feldman, & Hanes, 1981). Consequently, high fat diets are often used either to support a greater obesity, or to determine the efficacy of hypothalamic lesions for producing finickiness.

3. Metabolic Changes

i. Obesity

Although obesity is often indexed by body weight, obesity is defined in terms of percentage body fat and, therefore, is more appropriately considered a metabolic component of the syndrome (Hetherington & Weil, 1940; Kennedy, 1950). Increases in carcass fat can be detected as early as 4 hours post-lesion (Slaunwhite, Goldman, & Bernardis, 1972). VMH rats have been reported to achieve a 6 fold increase in adipose tissue mass (Han & Young, 1963; Hetherington & Weil, 1940; Keesey & Powley, 1975). Adipose tissue in VMH rats can represent 45-60% of body weight at sacrifice (Hetherington & Ranson, 1940; Montemurro & Stevenson, 1957).

Obesity occurs largely as a result of fat cell hypertrophy rather than hyperplasia (Hirsch & Han, 1969). In rats, fat appears to accumulate in all depots including the subcutaneous, retroperitoneal, omentum and mesenteries, perirenal, and pericardial fat pads (Hetherington & Ranson, 1940; Broebeck et al., 1943; Hirsch & Han, 1969). Finally, enlargement of

the liver seen in VMH rats results from infiltration of the liver by fat (Kennedy & Pearce, 1958).

ii. Digestive Secretions

Other evidence for a major metabolic effect of VMH lesions is indicated by alterations in a variety of digestive activities including elevations in basal secretion of insulin (Hales & Kennedy, 1964; Han & Frohman, 1970), gastric acid (Ridley & Brooks, 1965; Weingarten & Powley, 1980), pepsin (Ridley & Brooks, 1965), gastrin (Chikamori, Fukushima, Yashamita, Sayto, Nishimura, & Mori, 1983), and the rate of gastric emptying (Duggan & Booth, 1986). The gastrointestinal tract of obese VMH rats is found, on autopsy, to be dilated and can be twice the weight of controls (Boebeck et al., 1943).

Hyperinsulinemia is a defining characteristic of the syndrome and has been the most extensively studied metabolic disturbance. Exaggerated basal insulin secretion has not been detected in the first two postlesion days (Han & Frohman, 1970; Louis-Sylvestre, 1971; Steffens, Morgenson, & Stevenson, 1972). However, an exaggerated insulin release to intravenous glucose has been demonstrated during this period (Louis-Sylvestre, 1971) and as early as 10 minutes postlesion (Berthoud & Jeanrenaud, 1977; Rohner, Dufour, Karakash, Le Marchand, Ruf, & Jeanrenaud, 1977). Basal hyperinsulinemia develops over time and is clearly demonstrated by day five postlesion (Han & Frohman, 1970; Slaunwhite, et al., 1972). Morphological changes in pancreatic islets accompany hyperinsulinemia. Increases occur in islet size (Coleman & Hummel, 1970; Han, Yu, & Chow, 1970; Kennedy & Parker, 1963; Martin, Konijnendijk, & Bouman, 1974; Powley & Opsahl, 1976)

and in the nuclear (Setalo, 1965) and granule size (Kennedy & Parker, 1963) of beta cells.

Basal hypersecretion of gastric acid was first noted by Ridley and Brooks (1965) and has been consistently demonstrated following VMH lesions (Inoue & Bray, 1977; Powley & Opsahl, 1974; Ridley & Cirpilli, 1967; Sawchenko & Gold, 1981; Weingarten & Powley, 1980; Weingarten, Chang, & McDonald, 1985; Weingarten & Parkinson, 1988). Like hyperinsulinemia, basal acid hypersecretion develops over the first few days postlesion (Weingarten & Powley, 1980). However, significant increases in hydrochloric acid output were detected by Ridley and Brooks (1965) on the same day following VMH lesions. The increased gastric secretion by VMH lesions is comprised not only of an elevated hydrochloric acid output, but also by elevated pepsin (Ridley & Brooks, 1965) and gastrin (Chikamori et al., 1983) output.

These reviewed changes in visceral activities in VMH rats have been interpreted to reflect a general disturbance of the control of digestive secretions by the autonomic nervous system. Specifically, the alterations in digestive secretions are considered to be part of a more general increase in parasympathetic (i.e. vagal) tone on visceral functions (Powley, 1977; Powley, Walgren, & Laughton, 1983; Weingarten et al., 1985). This interpretation is corroborated by demonstrations that elevated visceral secretion by VMH lesions can be eliminated surgically by vagotomy (Powley & Opsahl, 1974; Sawchenko & Gold, 1981) or pharmacologically by blocking cholinergic receptors (Weingarten & Parkinson, unpublished observations), which mediate the effects of vagal activity on the gut. One group has reported an increase in the electrical activity of efferent vagal nerves (Yoshimatsu, Niijima, Oomura, Yamabe, & Katafuci, 1984). While it

is not yet understood what actual change takes place in the parasympathetic nervous system, the concept of an exaggerated vagal tone is useful because it greatly simplifies discussion of the constellation of digestive changes produced by the lesion.

An increased parasympathetic tone is accompanied by a decreased sympathetic tone as indicated by lower salivary gland weights (Inouye, Campfield, & Bray, 1977), and impaired lipolysis (Nishizawa & Bray, 1978). VMH lesions also disturb the normal circadian rhythm of lipolysis - lipogenesis. VMH rats do not show the normal daylight change from lipogenesis to lipolysis (LeMagnen, Devos, & Laure-achagiotis, 1980) suggesting that sympathetically activated mobilization of lipids is eliminated by the lesion.

Altered digestive secretions in the VMH syndrome have been highlighted by evidence indicating a parallel secretion profile in human obesity. Basal plasma insulin levels, and insulin secretion in response to oral glucose, are elevated in obese humans by comparison to non-obese populations (Bray & Gallagher, 1975; Komorowski, 1977). Obese humans also display exaggerated vagally-mediated reflexes such as insulin release to the sight and smell of food (Sjostrom, Garellick, Krotkiewski, & Luyckx, 1980). Reflexive salivation to food cues is also exaggerated in obese humans (Klajner, Herman, Polivy, & Chhabra, 1981). In one case study of a five-year-old girl with leukemic infiltration of the hypothalamus, Heaney, Eliel, Joel and Stout (1954) noted a sudden onset of hyperphagia and weight gain prior to death. A duodenal ulcer was observed on autopsy, suggestive of gastric acid hypersecretion.

These metabolic and behavioural changes produced by VMH lesions are related in terms of their anabolic roles and therefore, their relative

importance in producing obesity is not apparent. The variety of anabolic adjustments suggests a variety of mechanisms by which VMH lesions could produce obesity. Theories of the etiology of VMH obesity have been concerned with resolving this issue.

## II. THE ETIOLOGY OF VMH OBESITY

### Hormonal Mechanisms

The first systematic analysis of the cause of VMH obesity was performed by Hetherington and Ranson (1939, 1940, 1941, 1942a, 1942b, 1942c). The accepted explanation of hypothalamic obesity at the time was provided by Frohlich (1901), who first documented a series of case studies in humans with tumours in the area of the base of the third ventricle. Such tumours invariably encroached on the pituitary gland, and were associated with a variety of metabolic disturbances such as obesity, reduced growth, hypothyroidism, and reproductive system abnormalities suggestive of hypophyseal dysfunction. Consequently, Frohlich argued that the syndrome of disturbances typically produced by basomedial hypothalamic lesions was a consequence of damage to the pituitary gland. Based on this hypothesis, the disorder was labelled Frohlich's syndrome, and often, was referred to descriptively as the "adiposogenital syndrome" (Smith, 1927).

With the aid of the Horsley - Clarke stereotaxic device, which permitted restricted damage to defined and localized brain areas, Hetherington and Ranson were able to produce hypothalamic lesions in rats that avoided the pituitary. The result of these experiments was to define a "reactive region" for the obesity that was remote from the pituitary in the area of the ventromedial nucleus of the hypothalamus (Hetherington &

Ranson, 1940, 1941, 1942b). Other research demonstrated that obesity was not produced by damage restricted to the pituitary gland (Hetherington, 1943; Hetherington & Ranson, 1939; Smith, 1927). Furthermore, hypophysectomy did not affect the development of obesity produced by VMH lesions (Hetherington, 1943; Hetherington & Ranson, 1942a). These findings indicated that VMH obesity was not mediated by the pituitary gland. Rather, Hetherington interpreted his findings to favour a neural, rather than hormonal, origin of hypothalamic obesity.

With the pituitary deficiency hypothesis of the VMH syndrome dispelled, the mechanism of hypothalamic obesity was an issue. While it was recognized that VMH rats were hyperphagic and hypoactive (Hetherington, 1941, 1942c), and that these disturbances could cause or contribute to obesity, Hetherington and coworkers dismissed the view that VMH lesions induced obesity by a behavioural mechanism on the basis of evidence derived from a few animals. Specifically, two observations contradicted a simple behavioural explanation of the syndrome. First, some rats appeared to become obese in the absence of overeating (Hetherington, 1941; Hetherington & Ranson, 1942c). Second, other rats with lesion-induced reductions in activity levels did not become obese (Hetherington & Ranson, 1941). Instead, it was argued that cells in the VMH influenced fat metabolism directly through unidentified neural pathways that were likely organized in a rostral - caudal orientation (Hetherington & Ranson, 1940, 1942a).

## Behavioural Theories

In spite of Hetherington and coworkers' arguments, subsequent studies of VMH animals found a correlation between overeating and weight gain that could not be ignored (Broebeck, et al., 1943; Stellar, 1954). Broebeck

(1946) noted the findings of Keller and associates that described an "enhanced appetite" following VMH lesions in dogs and cats (Keller, Hare, & D'Amour, 1932; Keller & Noble, 1935; Keller, Noble, & Hamilton, 1936). In a classic series of experiments, Broebeck, Tepperman, and Long (1943) marshalled the following evidence for a behavioural interpretation of the syndrome.

(1) Concomitant measurement of daily caloric intake and body weight indicated a strong correlation between weight gain and calories eaten in control and VMH rats. Furthermore, the disproportionate weight gain following VMH lesions corresponded closely to the percentage elevation in caloric intake in VMH rats relative to control animals.

(2) The onset of hyperphagia following VMH lesions was rapid, often occurring before animals had completely recovered from the anesthetic used for lesion surgery. In fact this immediate hyperphagia typically exceeded later levels of caloric intake and led to a dramatic and rapid weight gain in the first 24 hours post-lesion.

(3) Weight loss by forced fasting in obese VMH rats equalled the predicted weight of fat that would be lost if prefasting caloric requirements were being replaced by mobilization of endogenous lipids. This relationship implied that the prefasting level of body fat was supported entirely by the elevated caloric intake.

(4) Finally, when the caloric intake of VMH animals was restricted to control levels, inordinate weight gain was found to be either eliminated, or very small.

Broebeck recognized that VMH obesity could result from lesion-induced changes other than overeating. Moreover, the residual obesity that was observed in some VMH rats that were prevented from being hyperphagic

(Broebeck et al., 1943) might be caused by a reduction in energy expenditures. Since VMH rats were reported to be hypoactive (Hetherington & Ranson, 1942c), reductions in activity levels might cause obesity. Alternatively, obesity might result from a direct metabolic effect of the lesion. Consistent with the latter possibility was the finding that food restricted VMH rats had elevated respiratory quotients relative to control rats, suggesting that VMH rats had reduced fatty acid utilization (Broebeck, et al, 1943). This metabolic effect could produce obesity and explain the residual weight gain observed in food restricted VMH rats. Broebeck used the following arguments to dismiss these alternative explanations of the VMH syndrome.

Broebeck's interpretation that VMH obesity was primarily a function of overeating rather than inactivity was based on two findings. First, hypothalamic lesions that produce inactivity do not always produce obesity. Some of the rats that were noted to have reduced activity by Hetherington and Ranson (1941) and by Broebeck (1946), did not beome obese. Second, although VMH rats are hypoactive, their greater body weight necessitates a greater energy requirement. Thus, more sensitive measures of activity found VMH rats to expend as much, or more, energy than control rats (Brooks, 1946).

Broebeck argued that any residual obesity occuring in VMH rats that were prevented from overeating was likely an artifact of the restricted feeding schedule, rather than a direct metabolic consequence of the lesion. Specifically, on food restricted schedules in which VMH rats were provided with a food ration equivalent to the daily intake of controls, the hypothalamic rats consumed this ration within only a few hours. According to Broebeck, this gorging behaviour might produce metabolic changes in

favour of fat deposition. In an experiment that attempted to mimick the gorging behaviour of VMH rats in brain intact controls, Tepperman et al. (1943) trained control rats to eat their daily food ration in a 3 hour period. This regimen produced elevated respiratory quotients in the brain-intact rats. Therefore, this metabolic change, also observed in VMH rats, was likely the result of gorging, and not a direct effect of the lesion.

Broebeck further argued that it was unlikely that the residual obesity occurring in VMH rats on restricted feeding was caused by an increase in the efficiency of digestion. Previous studies on humans had failed to demonstrate any alteration in the efficiency of digestive processes (i.e. "specific dynamic action") under fasting conditions. This line of research had come to the conclusion that adjustments in digestion favouring adiposity do not take place during fasting.

Finally, a reduction in energy expenditures (increased energy efficiency) by VMH lesions should be evidenced by a decrease in heat production. However, VMH rats had elevated rather than decreased heat production (Broebeck, et al., 1943).

Definitive experiments undertaken over two decades later demonstrated that the residual obesity that occurs in VMH rats prevented from overeating, is not a consequence of reduced activity levels or the pattern of eating (Han, 1967; Cox & Powley, 1981a) since it persists even in VMH rats that are tube-fed control amounts of food intragastrically, and when group differences in activity levels are eliminated. Consequently, this residual obesity is <u>now</u> considered to reflect a primary metabolic effect by hypothalamic lesions (Mayer & Thomas, 1967; Weingarten, et al., 1985).

However, Broebeck's arguments were influential for directing subsequent research on the VMH syndrome. Studies began to characterize the

hyperphagia of VMH animals in more detail in order to specify the motivational change caused by VMH lesions.

## The Satiety-deficit Theory

The first influential behavioural theory of the VMH syndrome considered VMH lesions to produce hyperphagia and subsequent obesity by an impairment in satiety. By this view, VMH lesions were considered to impair a mechanism, or mechanism(s) that normally terminated eating. The satiety-deficit theory was first suggested by Broebeck (1946) and elaborated as part of a more general theory of motivation by Stellar (1954). The major behavioural evidence for this model came from the observation that VMH rats increase their 24 hour caloric intake primarily by increasing meal size (Teitelbaum & Campbell, 1958).

This evidence for a role of the VMH in satiety was accompanied by observations suggesting that the lateral hypothalamus (LH) governed functionally opposite behaviours. LH lesions produced undereating and weight loss, while electrical stimulation of the LH could initiate feeding in a sated animal (Delgado & Anand, 1953). Consequently, Stellar (1954) suggested that the VMH and LH were motivational "centers" for satiety and hunger, respectively. Activity in the LH center was argued to activate eating, while activity in the VMH center suppressed eating by inhibiting the LH. Removal of the "satiety center" by VMH lesions, Stellar argued, produced an impairment in an animal's ability to terminate LH activation of eating. That the VMH was a satiety "center" was supported by electrophysiological data indicating that electrical stimulation of the VMH could terminate a meal in a feeding animal (Hoebel & Teitelbaum, 1966; Margules & Olds, 1962).

It was thought that the intact VMH might mediate satiety by monitoring the availability of essential nutrients, specifically, by metering the postprandial glucose rise. Several techniques for determining central glucose utilization rate suggested that the VMH had a high affinity for glucose (Mayer, 1953; Mayer & Marshall, 1956; Edelman, Schwartz, Cronkite, & Livingston, 1965a, 1965b; Panksepp, 1972). Such findings complimented generally accepted notions about the importance of the physiological availability of glucose in hunger and obesity (Mayer, 1955). Other studies demonstrated that VMH neurons respond to changes in the concentration of nutrients in the blood (Anand, Chhina, & Singh, 1962) and to visceral afferent activity (Anand & Pillai, 1967; Sharma, Anand, & Singh, 1961). These data could be taken to implicate the VMH as a site at which information regarding the availability of body nutrients was integrated to register satiety.

The satiety-deficit theory provided a simple explanation of VMH obesity that shaped research on the VMH syndrome for about 20 years. The theory was attractive because it integrated concepts that dominated thinking about hunger, satiety, and obesity with supporting physiological evidence. However, a long line of subsequent research has failed to support the theory. Specifically, although many attempts have been made to demonstrate a satiety deficiency induced by VMH lesions, VMH rats do not demonstrate any obvious disturbance in their ability to terminate eating to a variety of satiety producing agents. VMH rats respond normally to gastric distension (Smith, Salisbury, & Weinberg, 1961), infusion of nutrients into the stomach (Liu & Yin, 1974), duodenum (Novin, Sanderson, & Gonzalez, 1979) or bloodstream (Rowland, Meile, & Nicolaidis, 1975), and to gut hormones such as CCK 8 (Kulkoski, Breckenridge, Drinsky, & Woods, 1976)

and bombesin (West, Williams, Braget, & Woods, 1982) that suppress meal size in normal rats. They also make appropriate compensatory increases in intake to caloric dilution of foods (Smutz, Hirsch, & Jacobs, 1975; Thomas & Mayer, 1968). Furthermore, VMH rats maintain a hyperphagia, even when prevented from taking large meals, by increasing their meal frequency (Sclafani, 1978; Thomas & Mayer, 1978). These observations indicate that if VMH lesions disturb physiological mechanism(s) that normally regulate caloric intake, such a disturbance is not simply an inability to register satiety and affect meal termination.

## The Finickiness Hypothesis

In an alternative perspective of VMH hyperphagia, Teitelbaum (1955) argued that finickiness was the major behavioural disturbance produced by VMH lesions leading to overeating and weight gain. As already described, Teitelbaum (1955) demonstrated that VMH lesions produced an inordinate sensitivity to manipulations of diet palatability. VMH rats could be made to be more hyperphagic and to gain more weight by altering sensory aspects of their diet such as its taste and texture. Consequently, Teitelbaum suggested that the failure of VMH rats to maintain a normal caloric intake and body weight was due to a fundamental alteration in the ability of good tasting food to drive eating. This hypothesis contrasted with the satiety deficit theory in that it suggested that VMH obesity did not result from a failure to respond to postingestive consequences of food. Rather, VMH obesity occured because the gustatory consequences of food were more effective in eliciting eating.

Subsequent versions of the theory by other investigators broadened the concept of a sensory disturbance by VMH lesions to implicate a more

general overeactivity to environmental stimuli in VMH rats. Finickiness, or an increase in the affective response to taste, could be considered to be one behavioural marker of this general disturbance. Both empirical and anecdotal evidence described other behavioural disturbances by VMH lesions that supported the more general hypothesis. VMH rats were often noted to be overreactive to handling, hyperexcitable, and to display larger startle responses to extraneous stimuli (Panksepp, 1971). Such observations suggested an increase in "emotionality". These findings were interpreted by Grossman (1966) to indicate that VMH lesions exaggerate the animal's "affective responsiveness to sensory stimuli". Grossman's argument was also strengthened by the finding that VMH rats performed better in signalled avoidance tasks with shock as the reinforcer (US) (Grossman, 1966). This result would be expected if VMH rats were more sensitive to the stimuli used in the learning task.

Schachter (1971) used these and other observations to support a somewhat different view, termed the "externality" hypothesis of the VMH syndrome. Schachter drew a distinction between "external" environmental cues (e.g. sight of food) and "internal" physiological cues (e.g. satiety signals) that affect eating. According to Schachter (1971), VMH lesions resulted in an increase in the ability of "external cues" to elicit behaviours. He further reasoned that the strength of behavioural responses in VMH rats should be a function of the salience of the external cue. Finickiness was interpreted to reflect the exaggerated response to very salient, external taste cues. Schachter's hypothesis not only accounted for gustatory (i.e. finickiness) and emotional reactivity, but could also explain other behavioural observations. For example, VMH rats were reported to be less willing to work for food than controls. Specifically,

VMH rats eat less than controls when required to bar press for food on high fixed ratio reinforcement schedules (e.g. FR 256) (Teitelbaum, 1957) or when they are required to lift a weighted lid to obtain access to food (Miller, Bailey, & Stevenson, 1950). Schachter interpreted this apparently paradoxical lack of "motivation" to eat, to be a result of a failure to respond to more remote food related cues such as those occuring on high FR schedules, or when the food cup is covered.

Like the satiety-deficit theory, the finickiness hypothesis (and more general versions of this model) have encountered problems dealing with certain findings. Although it is generally accepted that VMH rats do overrespond to palatable foods and that this effect is a primary consequence of the lesion, VMH hyperphagia and obesity would not appear to be explained by finickiness or a more general overreactivity to sensory events.

Experiments testing the finickiness hypothesis have focused primarily on establishing the existence, and importance, of negative finickiness. It will be recalled that the finickiness of VMH rats is interpreted to reflect a disturbance in reactivity to the sensory qualities of foods. According to the finickiness hypothesis, VMH obesity results from the overresponding, by VMH rats, to palatable food (i.e. positive finickiness). The dependence of VMH obesity on finickiness was suggested by the finding that VMH rats reject unpalatable food (negative finickiness) and do not become obese on diets adulterated with quinine (which makes food taste bitter). However, demonstrations of negative finickiness may be explained by the aversive postingestive consequences of quinine. These postingestive events can be eliminated by sham feeding, in which rats are equipped with open gastric cannulae to permit the diet to drain out of the digestive tract. Using

this paradigm, it was found that VMH rats continued to be hyperphagic on adulterated liquid diet relative to controls over a wide range of quinine concentrations (Weingarten, Chang, & Jarvie, 1983).

Other observations suggest that negative finickiness may also be a function of obesity, rather than a direct consequence of the lesion. Bitter foods cause hypophagia only after VMH animals become fat (Ferguson & Keesey, 1975; Franklin & Herberg, 1974; Sclafani, Springer, & Kluge, 1976; Weingarten et al., 1983). Even in the original demonstration of finickiness, Teitelbaum reported that obese VMH rats were less hyperphagic on powdered diet than pellets, but were equally hyperphagic on these diets during the dynamic phase (i.e. before becoming obese) or when they were held at normal body weights. Therefore, reducing dietary palatability does not prevent overeating when VMH animals are not fat. Since overeating persists when negative postingestive consequences of foods are eliminated, and is insensitive to manipulations of diet palatability when VMH rats are lean, finickiness would appear to provide an insufficient explanation of VMH hyperphagia.

Despite the failure of the finickiness hypothesis to account for hyperphagia, positive finickiness would appear to be a primary component of the syndrome and not simply the result of obesity. Sham feeding VMH rats, held at control body weights by restricted feeding, continue to demonstrate a marked overreactivity to manipulations of diet palatability relative to controls (Weingarten, 1982).

Evidence counter to theories that view finickiness as a general increase in affective reactions (Grossman, 1966) or reactivity to external cues (Schachter, 1971) have also been presented. Early demonstrations of the failure of VMH rats to work for food may be explained by: (a) an

impairment on learning or memory by the lesion or, (b) by the effect of obesity on motivation to work for food. Rats who are trained to bar press for food prior to VMH lesions display no reduction in bar pressing for food relative to controls (King & Gaston, 1973). VMH rats prevented, by restricted feeding, from becoming obese will work as hard, if not harder, for food than controls (Beatty, 1973; Kent & Peters, 1973).

In summary, VMH hyperphagia does not appear to result from a disturbance in satiety. Furthermore, it is clear that the VMH rat overeats and is finicky. However, although finickiness contributes to obesity, it is not a sufficient explanation of hyperphagia and weight gain. It is possible that further research may elucidate a single behavioural variable disturbed by VMH lesions that might consolidate seemingly disparate observations. It is also possible that separate behavioural mechanisms may contribute to VMH obesity.

## Limitations of Behavioural Explanations for Metabolic Changes

More definitive evidence against behavioural theories of VMH obesity is provided by experiments demonstrating the persistence of obesity, and other metabolic changes, even when overeating is prevented by restricted feeding procedures. Han (1967) pair-fed VMH rats with controls by gavage, in which liquid diet was delivered directly into the stomach through oro-gastric tubes, to eliminate group differences in calories eaten and the pattern of eating. VMH lesion rats continued to fatten relative to controls with this procedure. The same effect occurs when animals are chronically pair-fed intragastrically and housed in cages that restrict motor activity to a minimum (Cox & Powley, 1981; Powley & Opsahl, 1974).

Therefore the residual obesity that occurs in VMH rats that are prevented from overeating is not caused by differences in activity, or more subtle feeding differences such as altered meal patterns as suggested by Broebeck (1946). This evidence argues in favour of a primary effect of VMH lesions on body fat depots. The term "metabolic obesity" was suggested by Mayer and Thomas (1967) to refer to this ability of VMH lesions to produce obesity independent of changes in behaviour, especially overeating. This term is used in the remainder of this thesis to refer to the obesity that is evident even in hypothalamic lesion animals maintained on restricted feeding regimens.

Restricted feeding procedures have also been important for determining whether altered digestive activities are primary consequences of VMH lesions or simply the result of hyperphagia. Conceivably, hypersecretion in a VMH rat could result from the increased load on the viscera caused by overeating (Assimacopoulos-Jeannet & Jeanrenaud, 1976). It is now clear that elevated visceral secretions occur in VMH rats even when hyperphagia and body weight gain are prevented by restricting caloric intake or weight gain to control levels. VMH rats continue to demonstrate a hyperinsulinemia even when their daily caloric intake is restricted to that of control rats (Goldman, Bernardis, & Frohman, 1974; Martin, et al., 1974), when they are tube-fed normal amounts of food (Han & Frohman, 1970) or when they are provided with a daily food ration sufficient to maintain their weight gain at control levels (Weingarten et al., 1985). Similarly, VMH rats that are held at the body weight of control rats continue to demonstrate elevated basal gastric acid secretion (Ridley & Brooks, 1965; Weingarten & Powley, 1980; Weingarten et al., 1985; Weingarten & Parkinson, 1988).

The primacy of metabolic disturbances following hypothalamic lesions has also been supported by studies on weanling rats. Weanlings do not demonstrate a spontaneous hyperphagia following VMH lesions. However, such lesions produce both hyperinsulinemia and an increase in carcass fat (Bernardis & Frohman, 1971). These data indicate that overeating is not necessary for obesity and implicate hyperinsulinemia more strongly in the primary obesity effect. Overall, the presence of these metabolic changes in the absence of hyperphagia indicates that behavioural theories do not provide a sufficient explanation of these components of the syndrome.

## Metabolic Theories

Metabolic theories suggest that one or more peripheral metabolic alteration, such as hyperinsulinemia, is(are) the primary consequence of the lesion which, in turn, causes both obesity and hyperphagia. Metabolic perspectives of the VMH syndrome were precipitated by a number of factors. First, behavioural studies failed to delineate a behavioural mechanism of overeating. Second, the work of Han (1967), Brooks (1965), and Frohman (Frohman & Bernardis, 1968, 1969) demonstrated that obesity and other metabolic disturbances such as elevated gastric acid and insulin secretions were independent of overeating. Therefore, metabolic disturbances could not be attributed entirely to hyperphagia. Finally, a number of key observations implicated hyperinsulinemia in the etiology of the syndrome. Insulin had long been recognized to be an important hormone of fat storage and glucose utilization. Insulin was linked, empirically, to eating behaviour and obesity by demonstrations that relatively large dose insulin injections activated eating (Booth & Brookover, 1968; Smith & Epstein,

1969; Steffens, 1969) and repeated insulin injections could produce obesity (Hoebel & Teitelbaum, 1966). These findings, in conjuction with the shortcomings of behavioural theories, prompted Powley (1977) and Bray and York (1979) to articulate metabolic theories of the VMH syndrome.

Metabolic theories are now numerous and motivate most of the present research on the syndrome. Fortunately, the metabolic theories of Powley (1977) and of Bray and York (1979) and other metabolic hypotheses (Duggan & Booth, 1986; Bray & Campfield, 1975; Frohman, 1978a,b; LeMagnen, 1983; Woods, Decke, & Vasselli, 1974) are related in that they focus primarily on VMH lesion-induced changes in the autonomic nervous system. These ideas differ only in the specific digestive response(s) argued to explain the syndrome.

It will be recalled that VMH lesions produce elevations in digestive activities interpreted as an elevated parasympathetic tone, and impairment of sympathetic responses suggestive of decreased sympathetic tone. Powley (1977) suggested, initially, that elevated parasympathetic tone was the primary disturbance produced by VMH lesions. Bray and York (1979) argued that the combined effects of elevated parasympathetic tone and decreased sympathetic tone were necessary to completely account for hyperphagia and obesity. Hyperinsulinemia was considered by both theories to be the major physiological alteration that mediated metabolic obesity and overeating. These two major theories are described in turn.

Powley's theory, termed the "cephalic phase hypothesis", suggested that VMH lesions exaggerated the cephalic phase of digestion by increasing central nervous system tone on the parasympathetic nervous system (i.e. the vagus nerve). Cephalic phase digestive responses are reflexes that are activated by receptors in the brain and oropharynx with stimulation by

sensory (as opposed to visceral) contact with food early in the feeding sequence. The vagus nerve is the major common efferent limb of these reflexes. Cephalic phase digestive responses are believed to prepare the digestive tract for the impending food (Valenstein & Weber, 1965; Nicholaidis, 1969).

Two concepts are important in Powley's scheme. First, the magnitude of the cephalic phase secretions are a function of the sensory quality of foods. Foods that are prefered in man and animals elicit larger cephalic phase secretions (Louis-Sylvestre, 1976; Louis-Sylvestre & LeMagnen, 1980; Wooley & Wooley, 1973; Janowitz, et al., 1950). Second, an exaggerated cephalic phase digestive response, particularly insulin secretion, would be expected to enhance digestion and metabolism (e.g. enhanced glucose storage) and thereby, produce secondary effects on behaviour and adiposity. To counteract the rapid incorporation of nutrients into tissues, more calories would need to be consumed and therefore feeding would be sustained for a longer period of time. In the absence of overeating, exaggerated digestive responses would have a direct metabolic effect favouring accumulation of fat. Based on these assumptions, Powley suggested that the cephalic phase hypothesis could explain; hyperphagia, finickiness, and metabolic obesity. Hyperphagia was a consequence of the activation of eating by the central nervous system when it detected activation of the cephalic phase reflexes. Since intake was presumed to be directly related to the magnitude of the cephalic phase response, palatable foods, which produce larger cephalic reflexes, would be expected to produce finickiness. Finally, even when hyperphagia was prevented, obesity resulted from the metabolic consequences of exaggerated digestive responses to food.

Bray and York's "autonomic hypothesis" suggested that VMH lesions altered the balance of parasympathetic and sympathetic "outflow" such that vagally mediated responses were larger while sympathetic responses were reduced. As already described, VMH lesions had been reported to reduce or eliminate sympathetically controlled variables such as salivary gland weights (Inoue, Bray & Mullen, 1977) and stress-induced lipolysis (Nishizawa & Bray, 1978). Few attempts have been made to determine whether impaired lipolysis is independent of other lesion effects such as hyperphagia, hyperinsulinemia, or obesity. However, a primary effect on lipolysis is supported by two findings. First, adult VMH rats lesioned as weanlings display elevated adipocyte responsiveness to insulin in the absence of hyperphagia or hyperinsulinemia (Goldman & Bernardis, 1974 a,b; Goldman, Bernardis, & Frohman, 1972). Second, the following observation made by Nishizawa and Bray (1978) strongly favours a direct influence of the VMH on sympathetic responses. Unilateral abdominal sympathectomy was demonstrated to reduce free fatty acid mobilization (FFA) during fasting from the denervated retroperitoneal fat pad relative to the contralateral intact pad in brain-intact rats. A similar effect could be produced by a unilateral VMH lesion, which reduced fasting induced FFA mobilization from the fat pad contralateral to the lesion. Bray and York suggested that the impairment in lipolysis by VMH lesions would be expected to promote adiposity by preventing the utilization of fats once they are stored. Thus, reduced sympathetic tone may promote lipogenesis by a direct metabolic route. Furthermore, since sympathetic activity inhibits insulin secretion (Woods & Porte, 1974, 1978), reduced sympathetic activity should exaggerate plasma insulin levels. This exaggerated hyperinsulimia would be

expected to contribute to the presumed effects of elevated insulin levels on eating and thereby also promote obesity by a behavioural route.

Metabolic theories, that consider elevated parasympathetic tone to account for the major behavioural and other metabolic effects of VMH lesions have been supported by a number of key findings:

1) Hypersecretion of insulin, the peripheral metabolic change considered most likely to cause the syndrome, occurs very early post-lesion (Steffens, et al., 1972; Tannenbaum, Paxinos, & Bindra, 1974). This early effect of the lesion on insulin secretion is consistent with the hypothesis that hyperinsulinemia is a primary effect of the lesion.

2) A variety of techniques for eliminating parasympathetic neural control over the viscera also reverse or prevent the syndrome. Vagotomy is reported to eliminate hyperphagia and excessive weight gain following VMH lesions (Powley & Opsahl, 1974; Inoue & Bray, 1977). Vagotomy also blocks hypersecretion of gastric acid and metabolic obesity (Cox & Powley, 1981b; Powley & Opsahl, 1974). Moreover, transplanting pancreases of controls into VMH rats who have their pancreases removed, also blocks hyperinsulinemia, hyperphagia, and weight gain (Inoue, Bray, & Mullen, 1978).

3) The magnitude of hyperinsulinemia early post-lesion is a good predictor of the eventual degree of hyperphagia and weight gain (Hustvedt & Lovo, 1972).

Metabolic theories of the VMH syndrome have also influenced research and thinking in the area of human obesity. Obese humans have been reported to have symptoms that parallel those of VMH rats, including increased basal digestive secretions and exaggerated cephalic phase digestive responses (Klajner et al., 1981; Sahakian, Lean, Robbins, & James, 1981; Sjostrom et

al., 1980). It has been argued that the metabolic profile of obese humans is the cause of their inability to control appetite and maintain normal levels of body fat (Sjostrom et al., 1980).

The cephalic phase and autonomic hypotheses are not sufficient explanations of the VMH syndrome. In an early test of the cephalic phase hypothesis, Cox and Powley (1981a) bypassed the cephalic phase digestive responses in VMH rats and controls by chronic intragastric tube feeding. VMH rats were found to have still accumulated a greater percentage carcass fat at sacrifice relative to controls. Therefore, metabolic obesity persisted in the absence of the cephalic phase of digestion. Subsequent studies found that elimination of anticipatory (i.e. cephalic phase) digestive reponses by pharmacological blockade with atropine did not block finickiness by VMH lesions (Sclafani & Xenakis, 1981). Finally, VMH lesions do not produce a consistent exaggeration of cephalic reflexes. Sham feeding-elicited gastric acid (Weingarten & Powley, 1980) and insulin (Louis-Sylvestre, 1976) secretions are elevated relative to controls. However, other procedures for producing cephalic phase responses have found VMH lesions to impair these reflexes. Specifically, rats trained by Pavlovian conditioning to expect a meal in a distinctive environment display cephalic phase digestive secretions in anticipation of the meal. VMH lesions eliminate, rather than exaggerate, anticipatory gastric acid (Weingarten & Parkinson, 1988 - see Appendix A) and insulin (Storlien, 1985) secretion. Furthermore cephalic phase gastric acid secretion, stimulated pharmacologically by 2 deoxy-D-glucose or low dose insulin is elevated but responses to high dose insulin are impaired relative to control rats (Weingarten & Parkinson, 1988). Therefore, it is as

reasonable to consider the role of reduced cephalic responses in VMH obesity as it is to consider the role of exaggerated digestive responses (Storlien, 1985; Weingarten & Parkinson, 1988). It remains possible, however, that exaggerated cephalic phase reflexes make some contribution to the overeating and obesity resulting from VMH lesions.

The "autonomic hypothesis" also appears to provide an inadequate account of the VMH syndrome. Specifically, reduction in sympathetic tone would appear to contribute little to the hyperphagia and obesity produced by VMH lesions. Chemical sympathectomy by guanethidine does not increase food intake or weight gain in brain-intact rats and does not exaggerate these changes in VMH lesioned rats (Powley, et al., 1983). The finding that vagotomy completely eliminates disproportionate adiposity in VMH rats pair fed with controls (Powley & Opsahl, 1974) could be interpreted to indicate that elevated parasympathetic tone is a sufficient explanation of metabolic obesity. However, vagotomy produces traumatic changes in eating behaviour and digestive processes and therefore, the latter result does not clearly rule out a role for reduced sympathetic tone in the metabolic obesity.

In addition to specific tests of the cephalic phase and autonomic hypotheses, a number of other observations now indicate that a metabolic perspective of the VMH syndrome is limited. The relevant findings are reviewed by Sclafani and Kirchgessner (1986) and can be summarized as follows:

1) Vagotomy eliminates hypersecretions but does not eliminate hyperphagia if animals are fed a very palatable high fat diet (Sclafani, Aravich, & Landman, 1981), and a residual hyperphagia (20%) remains even on less palatable diets such as regular lab chow (Inoue, et al., 1978).

2) Hyperinsulinemia is not a sufficient precondition for hyperphagia. Although large doses of insulin do stimulate food intake (Steffens, 1969), attempts to simulate chronic hyperinsulinemia by intravenous infusion of insulin have found hypophagia, not hyperphagia (Vanderwheele, Pi-Sunyer, Novin, & Bush, 1980).

3) Selective transection of coeliac vagal branches reduces hyperphagia, whereas sectioning hepatic or gastric branches does not (Sawchenko & Gold, 1981). However, selective gastric vagotomy reduces vagally-mediated insulin release more than coeliac vagotomy (Berthoud, Niijima, Sauter, & Jeanrenaud, 1983).

The evidence for and against metabolic theories indicates that altered autonomic responses may contribute to VMH obesity but are not a sufficient explanation of the overeating associated with the syndrome.

## III. CONCEPTUAL MODELS FOR UNDERSTANDING THE VMH SYNDROME

The mechanism by which VMH lesions produce both behavioural and metabolic consequences is fundamental to an understanding of the etiology of the syndrome and remains an unresolved issue. Historically, theories of the etiology of VMH obesity have been single mechanism models which attempt to reduce the constellation of lesion effects to a single primary disturbance, either behavioural or metabolic, that in turn causes secondary changes that characterize the syndrome. An important inadequacy of these "single mechanism" theories is their inability to account for the magnitude and variety of behavioural and metabolic components of the syndrome.

Since most early theories were generated within the framework of a behavioural analysis of the VMH syndrome, many of the studies that tested these theories evaluated their ability to explain behavioural findings rather than metabolic ones. As already discussed, these theories cannot explain metabolic obesity and primary effects of lesions on autonomic responses, and are limited even in their ability to explain behavioural findings. The more recent metabolic theories have resulted in a shift in the presumed role of the VMH in energy balance away from a motivational center in favour of a modulator of peripheral physiological processes. However, these theories are now recognized to be limited in their ability to explain overeating.

## The Dissociative Perspective

According to single mechanism theories, lesions of the VMH are presumed to disrupt a single system that controls either behaviour or metabolism. The manifestation of other syndrome effects is explained as the result of the interdependency of these processes outside the VMH. An alternative to a primary - secondary analysis of the syndrome is to view the coincidence of behavioural and metabolic disturbances as the result of a simultaneous disruption of separate mechanisms in the area of the VMH. VMH lesions are relatively large. The parameters affecting lesion size were determined by their ability to produce obesity (Hetherington & Ranson, 1939; Weingarten & Powley, 1980) and not by their ability to produce specific damage to anatomical substrates. The possibility that the size of VMH lesions may account for the disruption of both metabolic and behavioural processes has been acknowledged (e.g. Grossman, 1984; Powley et

al., 1980; Sclafani, 1971; Sclafani & Kirchgessner, 1986). For example, Grossman (1984) states that "It seems likely that the typical, large lesion that destroys both the ventromedial nucleus and surrounding tissue may in fact, produce several independent but potentially interacting symptoms including not only hyperreactivity to taste but also hyperphagia and hyperinsulinemia" (p. 8). Furthermore, Sclafani has suggested that, although damage to VMN does not appear to cause the overeating characteristic of the VMH syndrome, such damage may produce some of the metabolic consequences of larger VMH lesions (Sclafani, 1971; Sclafani & Kirschgessner, 1986). However, despite a recognition of this possibility, the hypothesis has never been clearly advocated as an alternative model of the VMH obesity syndrome until recently by Weingarten, who has suggested a "dissociative" perspective of the VMH syndrome. The "dissociative" model follows a series of experiments that compared behavioural and metabolic effects of VMH lesions with lesions of the paraventricular hypothalamus (PVH). Both VMH and PVH lesions were found to produce hyperphagia and an increase in percentage carcass fat when rats were fed ad libitum. However, when VMH and PVH rats were maintained at control body weights, only VMH lesions produced hyperinsulinemia, increased gastric acid secretion, and increased carcass fat. Weingarten interpreted these data to indicate that two systems involved in the regulation of body fat exist in the area of the VMH. One system influences food intake and is disturbed by PVH lesions. A separate system is involved in metabolism. VMH lesions disturb both behavioural and metabolic systems because such lesions are large and the two systems are in close anatomical proximity.

The dissociative perspective is attractive for a number of reasons. First, the etiology of VMH obesity has not been explained by a single

mechanism theory. No such theory has been able to account for the magnitude, or variety, of other effects of the lesion. Second, experiments on PVH rats and other studies of the anatomy of the syndrome have, to a certain extent, produced some of the syndrome disturbances in the absence of others. Indeed, a third attractive quality of the theory is that it forces a consideration of the relationship between the anatomy of the syndrome and its etiology, issues that are obviously related but seldom given equal consideration in the same theoretical and experimental analyses.

In the dissociative view, the manifestation of multiple lesion effects is explained as a consequence of the disturbance of separate mechanisms within the VMH. It follows that separate behavioural and metabolic effects could be demonstrated by more discrete ablation techniques. Evidence of this type can be extracted from studies of the anatomical mechanisms of metabolic and behavioural components of the VMH syndrome. This line of evidence is reviewed below.

## III. EVIDENCE FOR A DISSOCIATIVE THEORY

It could be argued that the failure of single mechanism theories to explain the VMH syndrome is reason enough for entertaining more complex formulations. However, as already indicated, a number of studies employing more discrete lesion techniques have provided more compelling evidence for a dissociative perspective. First, knife cut studies have produced hyperphagia without changes in parasympathetic responses (reviewed below). This research implicates a longitudinal fibre system that passes medial to the ventromedial hypothalamic nucleus (VMN) in the VMH area, as being

responsible for inhibiting feeding. Second, experiments that have restricted lesions to VMN have found that such lesions do not result in hyperphagia, even on palatable diets. There is some indication, however, that VMN is involved in the metabolic components of the syndrome.

## Knife Cut Studies

Knife cuts are performed to interrupt specifically communication between brain areas, unlike electrolytic lesions which destroy an entire brain area. Knife cuts were first performed to determine the direction by which the ventromedial hypothalamus might exert control over food intake. In a series of experiments, Sclafani and coworkers (Sclafani & Berner, 1977; Sclafani & Grossman, 1969; Sclafani & Maul, 1974), delineated a longitudinal fibre system responsible for inhibiting food intake. According to their analysis, this fibre system originates at the PVN and takes a descending course, turning laterally just rostral to VMN, passing between the VMN and the fornix, and terminating at, heretofore unidentified, caudal loci. Parafornical hypothalamic knife cuts are deemed to be effective for producing hyperphagia primarily because they sever fibres in this system at their lateral-medial orientation just rostral to VMN.

It has been suspected that this system terminates at the dorsal motor nucleus of the vagus (DMN) and/or the nucleus of the solitary tract (NST) since other behavioural and physiological evidence links these hindbrain structures to taste and digestive processes, and because histochemical data has indicated that PVN neurons do have axons that terminate in these nuclei (Luiten, Ger Horst, & Steffens, 1987; Swanson, & Sawchenko, 1983). Very recent findings support this hypothesis. Kirschgessner and Sclafani (1988)

demonstrated that hyperphagia and weight gain could be produced in rats by a unilateral parafornical hypothalamic knife cut combined with a contralateral cut made either; in the ventrolateral pons, more caudally in the ventrolateral medulla, and finally, beneath DMN/NST complex itself. Since unilateral parafornical hypothalamic knife cut lesions (PFKC) alone produce little or no hyperphagia, this technique is considered to produce overeating by destroying, bilaterally, only those fibres that form part of the same longitudinal feeding system. The possibility that these asymmetrical lesions actually disturb a bilateral fibre system originating in PVN, was supported in a subsequent study by the same investigators. Kirschgessner, Sclafani, and Nilaver (1988) found that PVN cells were labelled by Horseradish Perioxidase (HRP) when HRP was applied to the region of hyperphagia-producing coronal knife cuts in the ventrolateral pontine reticular formation.

Although the knife cut technique was adopted to study the direction of hypothalamic control over feeding, evidence from these studies has also become important in the analysis of the etiology of the syndrome. Measurement of metabolic consequences of knife cuts were first reported to be similar to classic VMH lesions. Knife cut rats were found to have elevated basal insulin (Sclafani, 1981) and gastric acid secretions (Sawchenko & Gold, 1981). However, these measurements were performed on rats that were permitted to overeat. Subsequent reports have found an absence of hyperinsulinemia following knife cuts in rats that are prevented from overeating (Bray, Sclafani, & Novin, 1982; Sclafani, 1981). Other findings also indicate that hyperphagia-producing hypothalamic lesions do not invariably produce metabolic changes seen in VMH lesion animals. Lesions of the paraventricular hypothalamus have also been found to produce

no change in digestive secretions when hyperphagia is prevented (Weingarten et al., 1985). Furthermore, PVH lesions produce body compartment changes in favour of increased percentage fat when rats are permitted to overeat, but not when they are held control body weights (Weingarten et al., 1985). These findings, in combination, have been argued to indicate that VMH hyperphagia and obesity are not explained by altered parasympathetic tone, and that these two lesion techniques interrupt a common system that is involved in the control of food intake but not metabolism (Sclafani & Kirchgessner, 1986).

These data support a dissociative perspective of the VMH syndrome. However, restricted feeding procedures have not invariably eliminated hyperinsulinemia in PFKC rats. It was initially reported by Tannenbaum et al. (1974) that hyperinsulinemia persisted in PFKC rats held at control body weights. It is not clear what might account for this discrepancy between studies. The restricted feeding procedure used by the latter researchers may have been insufficient to entirely prevent overeating. It is also possible that the assay for insulin was sufficiently insensitive to result in the failure to detect hyperinsulinemia in the Bray et al. (1982) and Sclafani (1981) studies. Fasting reduces insulin levels, and therefore, differences in secretion under fasting conditions may be relatively difficult to detect. Therefore, the effect of PFKC lesions on parasympathetic tone is not yet clear.

Since increased parasympathetic tone is the most likely mediator of metabolic obesity, procedures that presumably produce no alteration in vagally controlled responses such as knife cuts or PVH lesions, should not produce obesity when excessive food intake and weight gain is eliminated. This hypothesis has been confirmed on the PVH lesion rat. However, body

compartment changes have not been evaluated following PFKC lesions. In summary, if PFKC lesions produce hyperphagia, and only secondarily affect metabolic changes, a number of predictions can be made. First, PFKC lesions should not produce changes in other indices of parasympathetic tone when hyperphagia is prevented. For example, gastric acid secretion has not been determined in food restricted PFKC rats. Although gastric acid secretion is not implicated as strongly as hyperinsulinemia in the etiology of the syndrome, it is easier to measure and is an excellent index of vagal activity. Second, if metabolic obesity is mediated by increased parasympathetic tone, PFKC lesions should not produce obesity when hyperphagia is prevented. Finally, if VMH and PFKC lesions produce hyperphagia, but only VMH lesions produce primary metabolic changes, then VMH lesions should produce a greater obesity than PFKC lesions when rats are fed ad libitum. Answers to these questions require not only the determination of the effects of knife cuts on metabolic variables that have never been documented, but also requires systematic comparisons between VMH and PFKC rats under the same experimental conditions to determine relative effects of these lesion on parasympathetic tone, overeating, and the final level of obesity achieved by these different ablations.

If fibres in the parafornical hypothalamic area are not responsible for the primary metabolic consequences of VMH lesions, then some other mechanism must control the metabolic changes. That this control may be exerted by the VMN, is suggested by studies that have restricted electrolytic lesions to the VMN, avoiding damage to other VMH areas.

Restricted VMN Lesions

The ventromedial hypothalamic nucleus (VMN) is a dense aggregate of cells within the ventromedial hypothalamic area (VMH). Although the nucleus is the target of VMH lesions, the perimeters of the ablation extend far beyond the nucleus itself. Consequently, the issue has been raised as to whether it is damage to the nucleus alone that is responsible for the VMH syndrome or whether surrounding areas are important. To address this issue, a small number of studies have been conducted in which lesions are focussed in the VMN and made smaller in order to minimize damage to surrounding tissue. In an often cited study by Gold (1973) it was found that small lesions, restricted to VMN, do not result in either hyperphagia or weight gain, even on a very palatable high fat diet. This finding has tended to direct interest away from the nucleus as an important site for energy regulation (Gold, 1973; LeMagnen, 1983; Sclafani & Kirchgessner, 1986). However, one hypothesis, consistent with a dissociative view, is that the VMN controls metabolic processes, possibly parasympathetic tone, and therefore that destruction of the VMN may produce disturbances in digestive secretions and a metabolic obesity.

A study by Bernardis and Frohman (1971) on weanling rats produced results consistent with this idea. By making small lesions in the vicinity of the VMN, these researchers attempted to localize the specific site for production of metabolic disturbances. They found that only those lesions that were centered in the VMN produced hyperinsulinemia and an increase in percentage body fat. Although this result supports a role for the VMN in VMH obesity, it is not a clear demonstration of the dissociation of behavioural and metabolic disturbances for the following reasons. First, the restricted VMN lesions were large enough to produce significant damage

to surrounding areas and therefore, it is not clear that damage to the VMN per se accounts for the metabolic effects. Second, larger VMH lesions produce hyperinsulinemia and obesity but not hyperphagia in weanling rats (Bernardis & Frohman, 1970). From a dissociative perspective, it is not only important to demonstrate that restricted VMN lesions produce metabolic effects, but also that they do not produce overeating. It is therefore, important to evaluate the effects of VMN lesions in adult animals in which the larger VMH lesions normally produce hyperphagia.

## RESEARCH APPROACH

Clearly, much evidence already exists for a dissociative perspective of the VMH obesity syndrome. However, certain questions that are central to a dissociative analysis remain unanswered. They include:

1) Do PFKC lesions produce an obesity as defined by increased percentage body fat? Although it has been shown that PFKC rats are hyperphagic and gain weight, it is not known whether they become truly obese as do VMH rats. The previously described findings that increases in body fat can occur in hypothalamic lesion animals independently of changes in body weight, indicate that weight gain does not always reflect the level of obesity. Since the degree of obesity produced by hypothalamic lesions is accurately determined by measurement of percent body fat, not weight gain, it is important to determine the effects of knife cuts on body compartment changes.

2) Do PFKC lesions produce metabolic disturbances such as increased parasympathetic tone and obesity in the absence of hyperphagia?

3) Do VMN lesions, which presumably do not produce hyperphagia, result in an increased parasympathetic tone and/or obesity?

To address these questions, a dissociative analysis of the VMH syndrome was undertaken in which an attempt was made to selectively affect behavioural or metabolic systems by using knife cuts and restricted VMN lesions. The effects of these ablations on metabolic and behavioural measures were compared with classic VMH lesions under both ad libitum feeding and restricted feeding conditions.

This approach, in which different ablation syndromes are systematically compared under the same experimental conditions, offers two important advantages over previous experiments. First, comparing each ablation technique with classic VMH lesions permits analysis of the independent contributions of behavioural and metabolic mechanisms to obesity. Second, comparison of selective hypothalamic lesions (i.e. PFKC vs VMN) in the same study would permit analysis of the relative effects of each lesion on behavioural and metabolic parameters. For example, even a very small VMN lesion that produces obesity, may still produce some hyperphagia due to the anatomical proximity of behavioural and metabolic mechanisms. However, dissociation of metabolic and behavioural disturbances would be indicated if VMN lesions produced relatively less overeating than PFKC lesions, but a relatively greater increase in parasympathetic tone or percentage body fat than knife cuts.

In summary, if VMH lesions do not disturb separate systems involved in eating, digestive secretions, and obesity, then PFKC lesions and VMN lesions will not be differentially effective for producing behavioural and metabolic disturbances. If VMN lesions are relatively more effective than PFKC lesions for producing metabolic disturbances but less effective for producing hyperphagia, separate systems for behavioural and metabolic components of the VMH obesity system exist.

## CHAPTER 2. GENERAL METHODS

### Experimental Subjects

The subjects were male Long-Evans hooded rats weighing between 270 to 370 grams at the time of surgery. Rats were obtained either directly from, or bred from stock obtained from, Blue Spruce Farms (Altamont, New York). Animals were housed individually in wire mesh hanging cages in a colony room at 25 degrees centigrade and on a 14:10 hr light/dark cycle. Water was available ad libitum and food was provided according to the specific experimental protocols.

### Surgery

a. Gastric Cannula Preparation :

To collect gastric secretions (Experiment 2) rats were implanted with chronically-indwelling stainless steel gastric cannulae. Animals were anesthetized with sodium pentobarbitol (Somnotol, 45mg/kg;intraperitoneal) during surgery and were food deprived for 24 hours prior to operating. The gastric cannula was an 11 mm stainless steel tube flanged at both ends (8.5 mm outside diam. X 7.9 mm inside diam.). Marlex mesh (2.5 cm.) was fixed around the shaft using dental cement. This technique, in conjuction with

healing processes, served to anchor the cannula in the abdomen.

To implant the cannula, a midline laparotomy was made through the skin and abdominal wall and the stomach was exposed. Two concentric purse string sutures, using 5-0 silk, were made in the wall of the forestomach and a fistula was made within this circle. One end of the cannula was fitted into the stomach and the sutures were drawn and tied. The remaining end of the cannula was pushed through a stab incision in the abdominal wall and skin, and a second piece of Marlex mesh was placed, subcutaneously, around the cannula. The entire apparatus was secured by a single purse string suture (3-0 silk) made in the skin surrounding the exteriorized cannula shaft. The abdominal wall was closed with interrupted sutures using 3-0 catgut, and the skin was closed with stainless steel wound clips.

b. Hypothalamic Lesions

All brain surgery was performed under sodium pentobarbitol anesthesia (45mg/kg) and followed by 24 hours food deprivation. To produce hypothalamic lesions, animals were placed in a Kopf stereotaxic instrument with the head positioned level. A 1.5 mm incision was made in the skin to expose the skull and two 1 mm holes were drilled bilaterally in the skull at coordinates required for the type of lesion. The following types of lesions were performed according to the different experimental conditions:

(1) Ventromedial Hypothalamic Lesions (VMH)

(2) Parafornical Hypothalamic Knife Cut Lesions (PFKC)

(3) Ventromedial Nucleus Lesions (VMN)

(4) Combined PFKC and VMN Lesions (PFKC/VMN)

(5) Sham Lesions (SHAM-L)

(6) Sham Knife Cuts (SHAM-KC)

(7) Combined sham lesions and sham knife cuts (SHAM-L/SHAM-KC)

The materials and procedures required to perform electrolytic lesions or knife cuts were as follows.

i) Electrolytic Lesions:

Bilateral electrolytic lesions were made to destroy either the ventromedial hypothalamus or the ventromedial nucleus of the hypothalamus depending upon the experimental condition to which a subject was assigned. The lesion parameters for VMH lesions reported by Weingarten and Powley (1980) were used to produce VMH lesions. The parameters for VMN lesions were determined empirically and based on their ability to produce visible damage to the entire medial-lateral extent of VMN, but not progressing beyond the lateral border of the nucleus. Since the nucleus is cylindrical, whereas lesions are round, lesions meeting this criterion did not produce visible damage to the entire anterior-posterior extent of the nucleus. This strategy was adopted for theoretical reasons. Since the putative hypothalamic fibre system involved in food intake is believed to pass just lateral to VMN, larger lesions would be more likely to disturb this system and produce hyperphagia.

Lesions were made using a Lesion Producing Device (Stoelting Co.) by passing anodal direct current through an electrode. The tail immersed in a saline bath served as the cathode. The electrode was made from a #00 stainless steel insect pin that was coated with Epoxylite except for 0.4 mm at the tip. Sham lesions involved the same procedure as VMH lesions except that no current was passed.

For VMH lesions, the coordinates were 1.3 mm posterior to bregma, .6 mm lateral to the midline, and 9.2 mm below the surface of the skull. A 1.0 mA current was passed for 17 seconds. The coordinates for VMN lesions were 1.0 mm posterior to bregma, 0.5mm lateral to the midline, and 9.5mm below the surface of the skull. A 1.0 mA current was passed for 8 seconds.

ii) Parafornical Hypothalamic Knife Cut Lesions:

The procedure for producing knife cuts was designed from the technique described by Sclafani and Grossman (1969). The knife consisted of a 6.5 mm long guide cannula, and tungsten wire sharpened at one end served as the blade. The guide cannula was made from stainless steel tubing (.46 mm outside diameter, .10 mm inside diameter) that was bent at a 45 degree angle, 2 mm from one end. To operate the knife, the tungsten wire, which normally remained retracted inside the cannula, was extended 3.5 mm out of the lower end of the cannula. The guide cannula was positioned using standard stereotaxic procedures. Therefore, the surgical procedure and determination of lesion coordinates for knife cuts was identical to VMH lesions except that cuts, rather that electrolytic lesions, were made.

To produce knife cuts, the guide cannula was lowered to the following coordinates; 0.6 mm anterior to bregma, 1.0 mm lateral to the midline, and 7.4 mm from the surface of the skull. The tungsten wire knife was then extended and the guide cannula was lowered 2.9 mm, producing a cut that extended ventrally to the base of the brain. Finally, the tungsten wire knife was retracted and the guide cannula was removed. The holes in the skull were filled with bone wax (Ethicon) and the skin was sutured closed with 3-0 silk. The knife was cleaned with 70% ethyl alchohol following

each cut. Sham cuts involved the same procedure except that the wire was not extended and the knife was not lowered beyond 7.4 mm below the surface of the skull.

Sacrifice

Rats were sacrificed with a lethal dose of sodium pentobarbitol (65 mg/kg). Each rat was perfused intracardially with 10% buffered formalin followed by .15M saline. The brain was removed and stored in buffered formalin for histology. In Experiments 1a and 2, the carcass was prepared for carcass fat determination. The carcass analyses and brain histology were conducted as follows.

a. Carcass Analysis.

The major purpose of carcass analysis was to obtain a measurement of the percent of the body constituted as fat (i.e. the level of obesity). The procedure used for determining percent carcass fat was that described by Cox, Laughton, and Powley (1985). At sacrifice, the head and tail were removed and the carcass was shaved. The gastrointestinal tract was rinsed with water to remove remaining contents and the carcass was frozen at -18 C until the time of analysis. To determine body fat composition, the carcass was quartered and completely dessicated in a drying oven at 60 degrees centigrade. Percentage carcass fat was estimated from the percentage body water using a regression equation that was derived, and determined to be a valid measure of level of body lipid in the rat, by Cox et al. (1984). The equation is: Percentage body fat = percentage body water x -1.272 + 95.963.

Prior to deriving this equation, percent body fat was typically determined by homogenizing the dessicated carcass, extracting body lipids using an organic solvent such as ether, and then evaporating the solvent leaving the pure body fat compartment. However, this procedure is impractical and requires the use of large volumes of highly flammable solvents. Since body lipid is stored in a relatively dehydrated form, the percentage body water reflects the level of body fat. Cox et al. (1984) found percentage body water to be a precise estimator of the percentage carcass fat. Using 373 animals from nine studies in which rats were rendered obese by different techniques, including various hypothalamic lesions, regression equations were derived for the relationship between percent water and percent fat determined by ether-extraction. Pearson product-moment correlation coefficients over the nine studies ranged from -.972 to -.995. The overall correlation coefficient was -.988. The equation shown above is the overall regression equation for the data pooled for all 373 animals. Percent water accounted for 97.7% of the variance in percent fat and the standard error of estimate of % fat was 2.38%. The function was found to be linear over a range of 6.6% to 68.8% body fat. Therefore, this equation provides an accurate and practical index of the level of obesity in the rat.

b. Histology

All brains with lesions were subject to histological analysis to determine the location and extent of neural damage. After being fixed in formalin and stored for a minimum of 1 month, brains were frozen and sectioned in the coronal plane in 40 uM sections. Sections were taken through the ventromedial hypothalamus and beyond the VMH when this was

necessary to identify the anterior or posterior aspect of the lesion or knife cut. The slices were mounted on microscope slides and stained with luxol fast blue and cresyl violet to identify fiber tracts and cell bodies, respectively. Final assignment of rats to groups was determined entirely by the results of histology. Evaluation of tissue damage was performed by a rater who was blind to the results of dependent measures.

VMH Lesions: The objective of VMH lesions was to remove, bilaterally, the area of the hypothalamus between the paraventricular nucleus anteriorly and the mamillary bodies posteriorly, and which extends from the third ventrical laterally to the fornix. To be considered successful lesions, and thereby be included in data analyses, VMH lesions had to remove the ventromedial nucleus. Since the center of lesions is variable, this criterion resulted in the acceptance of lesions that both removed the nucleus and considerable tissue in the surrounding VMH area. Successful VMH lesions typically encroached on the dorsal hypothalamus and could extend as far dorsally as the top of the third ventricle.

VMN Lesions: The boundaries for VMN were defined, and identified, by reference to the stereotaxic atlas of the rat brain provided by Konig & Klippel (1963). In the coronal plane, the anterior and posterior borders of VMN are located at A 5150u and A 3990u, respectively. The technology used for hypothalamic lesions and the shape of VMN make it impossible to perform lesions that would remove all or most of the nucleus without substantial damage to surrounding areas. The nucleus is cylindrical, slightly flattened, and tilted medially, whereas electrolytic lesions are spherical. In this thesis, the size of the lesion was determined initially using a series of lesions employing different lesion parameters, so as to restrict observable necrosis to the perimeter of the nucleus, thereby

leaving parts of the anterior and/or posterior nucleus intact. The rationale here was theoretical and based on the desire to avoid damage to fibres that might pass laterally to VMN and that may be involved in eating behaviour.

Due to the difficulties in determining: the exact perimeter of the VMN, restricting lesions to VMN, and the exact boundaries of lesion damage, it was not possible to define successful VMN lesions as those that were restricted only to VMN. Rather, animals were excluded from data analysis only when the center of the lesion was not located within the nucleus. This criterion proved to be satisfactory because the center of lesions were relatively easy to identify, and no animals had lesions that were centered near the boundary of the nucleus.

PFKC Lesions: PFKC lesions were included in data analysis when they extended bilaterally over the entire anterior-posterior extent of VMN, remained medial to the fornix, and did not visibly fall bilaterally within the VMN. Knife cut lesions were excluded from data analysis when: (a) the above criteria were not met, or, (b) dilation of the third ventrical suggested the possibility of unintended bilateral damage to the VMN.

Knife cuts that encroached on the lateral border of VMN but did not clearly lie within the nucleus, were considered successful and included in data analysis. Furthermore, since the medial-lateral distance between cuts was highly consistent, a cut that encroached on VMN unilaterally, was remote from VMN contralaterally. Occasionally, PFKC lesions produced dilation of the third ventricle in the direction of the cut. Dilation of the ventricle did not constitute reason for exclusion provided that the cut remained outside of the nucleus and the dilation was unilateral.

These criteria ensured that any unintended damage to the nucleus remained unilateral. This rule was considered reasonable because unilateral hypothalamic lesions have little, if any, effect on the dependent measures being studied (Gold, Quackenbush, & Kapatos, 1972; Sclafani, & Berner, 1977). Furthermore, any unintended damage by PFKC lesions to VMN would be expected to reduce the probability of dissociating behavioural and metabolic disturbances and, therefore, including animals with damage to VMN is a conservative error (i.e. PFKC lesions would produce undesired metabolic changes).

PFKC/VMN Lesions: Combined PFKC and VMN lesions were defined according their ability to fulfill the criteria for both successful PFKC and VMN lesions as defined above. The combination of lesions typically produced some dilation of the third ventricle that could have produced non-specific damage to surrounding brain areas. Rats were only excluded from analyses when: (1) knife cuts or lesions did not meet the criteria defined above, or (2) when dilation of the ventricle obscured the locus of lesions or knife cuts.

Measurement of Gastric Acid Secretion (Experiment 2)

Gastric secretion trials were conducted in 17 hour food-deprived rats. On test days, each rat was removed from its home cage and its cannula was opened by removing the set screw that normally kept it closed. The stomach was rinsed with .15M saline to remove any remaining food. A collecting tube, consisting of a 1.9 cm long stainless steel tube attached to a 15 cm length of Tygon tubing, was threaded into the cannula. The subject was then placed in a Plexiglas test cage (10.2 cm wide x 20.3 cm long x 10.2 cm high), that was mounted on 15.25 cm stilts. The center bars

of the cage floor were spaced 1.6 cm apart to allow the collecting tube to hang below the cage and permit the rat to move freely. Gastric secretions drained by gravity force down the collecting tube and into a plastic vial that was force-fitted onto the free end of the collecting tube.

Three parameters of gastric secretion were obtained: i) volume of secretion collected (expressed in mls/2hr); ii) acid output (expressed as uEq H+/2hr) and determined by automatic titration (Radiometer-Copenhagen titration unit) of the sample to pH 7.0 using .05 N NaOH, and; iii) acid concentration (expressed as uEq H+/ml) calculated by dividing the total acid output by sample volume.

Data Analysis

All data were analyzed by Analysis of Variance (ANOVA) with alpha = .05. When group by trials interactions occurred, F tests for simple effects were used to examine mean differences between groups within each trial, or between trials within experimental groups. Where warranted, post hoc multiple comparisons using the Newman Kuels procedure was undertaken to identify the source of simple effects.

Data for sham lesion and sham knife cut animals were compared on all dependent measures. Comparisons on group mean data for the dependent measures used, are shown in Appendix B. Control groups were not significantly different on any dependent measure. Therefore, data for sham lesion and sham knife cut control groups were pooled for data analyses.

# CHAPTER III. EFFECTS OF VMH, PFKC, AND VMN LESIONS ON AD LIBITUM CALORIC INTAKE, BODY WEIGHT, AND BODY FAT

## EXPERIMENT 1A

Gold (1973) reported that electrolytic lesions of VMN did not produce hyperphagia or weight gain in adult rats. However, no other published report exists showing that this effect can be replicated. Furthermore, since Gold did not determine body composition, it is not known whether VMN lesions affect body fat depots. Therefore, it is possible that damage to the VMN produces some of the metabolic effects associated with larger VMH lesions, such as obesity that occurs even on restricted feeding regimens (i.e. metabolic obesity). Although data on weanling rats suggest that damage to VMN may produce a metabolic obesity, the effects of restricted VMN lesions on body composition remain to be determined in mature rats. Therefore, the first hypothesis to be tested by this thesis is that lesions of VMN produce no overeating but continue to produce obesity.

A test of this hypothesis is a reasonable first step in a dissociative analysis for two reasons. First, no study exists showing that a metabolic effect of hypothalamic lesions can be produced without disturbing the system responsible for hyperphagia in adult animals. Support for a dissociative perspective requires that metabolic effects of hypothalamic lesions be produced without affecting the mechanism(s)

controlling food intake. Second, the phenomenon of metabolic obesity is the most important evidence for a pure metabolic component to the VMH obesity syndrome. Therefore, measurement of percent body fat following VMN lesions provides the most critical examination of the possible metabolic contribution made by VMN damage to the VMH syndrome.

Parafornical hypothalamic knife cut (PFKC) lesions have been reported to produce the hyperphagia and concomitant increase in body weight that are characteristic of VMH lesions (Sclafani, 1971). However, changes in body compartments have not been determined following PFKC lesions. Therefore, it is not known whether this ablation technique actually produces obesity. Furthermore, to determine the contribution made by PFKC lesion-induced overeating to the level of hypothalamic obesity, it is important to compare the relative effectiveness of PFKC and VMH lesions for increasing the amount of body fat. According to a dissociative perspective, PFKC lesions should produce less obesity than VMH lesions if such lesions do not produce the metabolic consequences that accompany VMH damage.

To address these issues, this first experiment compares the effects of VMH, PFKC, and VMN lesions on caloric intake, body weight, and body fat in animals feeding ad libitum. In addition, since the effect of VMH lesions on caloric intake and body weight depends on the type of diet used, the present experiment also employs a series of test diets previously shown to produce different degrees of hyperphagia and obesity in hypothalamic lesion rats (Sclafani & Berner, 1977; Weingarten, et al., 1985). This procedure was included to ensure that any group differences observed were not simply specific to one test diet.

# METHODS

## Protocol

Rats were assigned to one of four experimental conditions (VMH, PFKC, VMN, SHAM) so that, at the beginning of the study, the groups would be similar in mean body weight and variance. Half of the SHAM animals received sham lesions, while the remaining half received sham knife cuts.

Following stereotaxic surgery, rats were deprived of food for 24 hours. For the next 66 days, rats were maintained on a series of test diets. The caloric composition of these experimental diets is presented in Table 1. The first was a nutritionally complete "Powder" diet

Table 1. Composition and Kcal value of Test Diets Used in Experiment 1a.

| DIET | COMPOSITION (by weight) | Kcal VALUE |
|---|---|---|
| Powder | - | 3.61 Kcal/g |
| Mash | 65% Water<br>35% Powder | 1.26 Kcal/g |
| High Fat | 67% Powder<br>33% Crisco Oil | 5.50 Kcal/g |

(Rodent Laboratory Chow #5001, Purina Mills). The second diet, termed "Mash" consisted of the powder diet mixed with water (65% water, 35% powder by weight). The third diet, termed "High Fat" consisted of the powder diet mixed with vegetable oil (67% powder, 33% Crisco oil by weight). The diet

was changed to Mash on day 22 and then to High Fat on day 44. The final recording of food intake was made on day 66, resulting in 22 days access to each of the three test diets.

Caloric intake and body weight were determined every second day over the experiment. Food cups were removed from cages and the weight of food eaten was measured by subtracting the weight of the cup and its remaining contents, from the original weight. The weight of diet consumed was converted to caloric intake by multiplying the weight by the caloric density of the diet indicated in Table 1.

## RESULTS

### Histological Findings

Determination of the locus of hypothalamic damage by brain histology was undertaken to select animals for inclusion into experimental groups. Rats with lesions that met the individual criteria described in General Methods for successful VMH, PFKC, or VMN lesions constituted the experimental groups used in omnibus data analyses. Of the 58 rats that initially had hypothalamic lesions, 1 rat did not recover from surgery as indicated by dramatic weight loss post-surgery. Two rats became ill (started losing weight dramatically) before the end of the experiment and were sacrificed. Eighteen animals had lesions that did not meet the required criteria. This left a total of 37 animals in the four experimental conditions with group sizes as follows: SHAM (n = 10), VMH (n = 9), PFKC (n = 10), and VMN (n = 8). The histological findings for animals included in data analysis are described below.

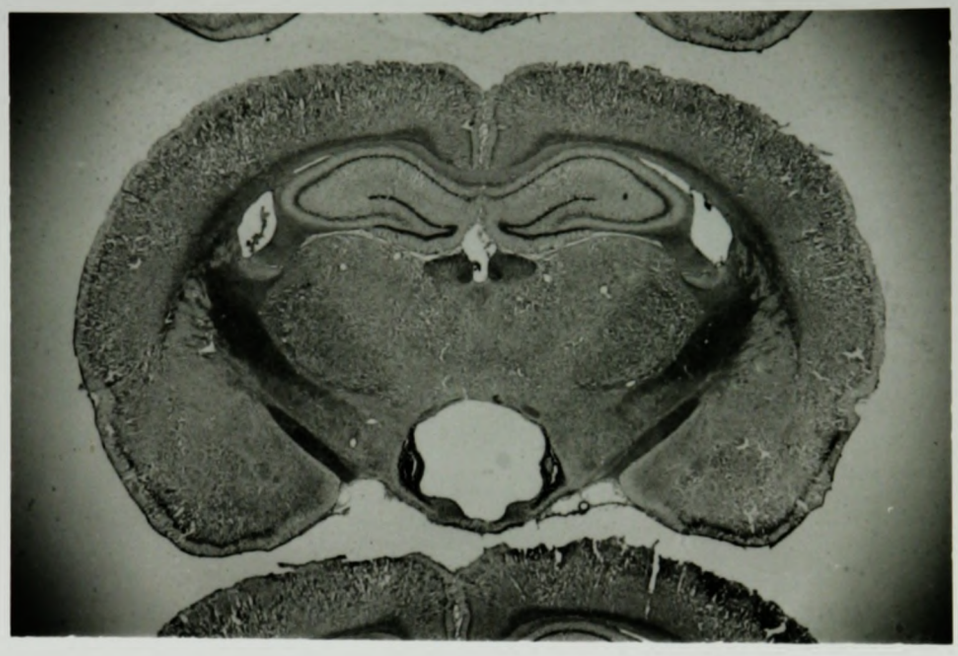
Ventromedial Hypothalamic Lesions

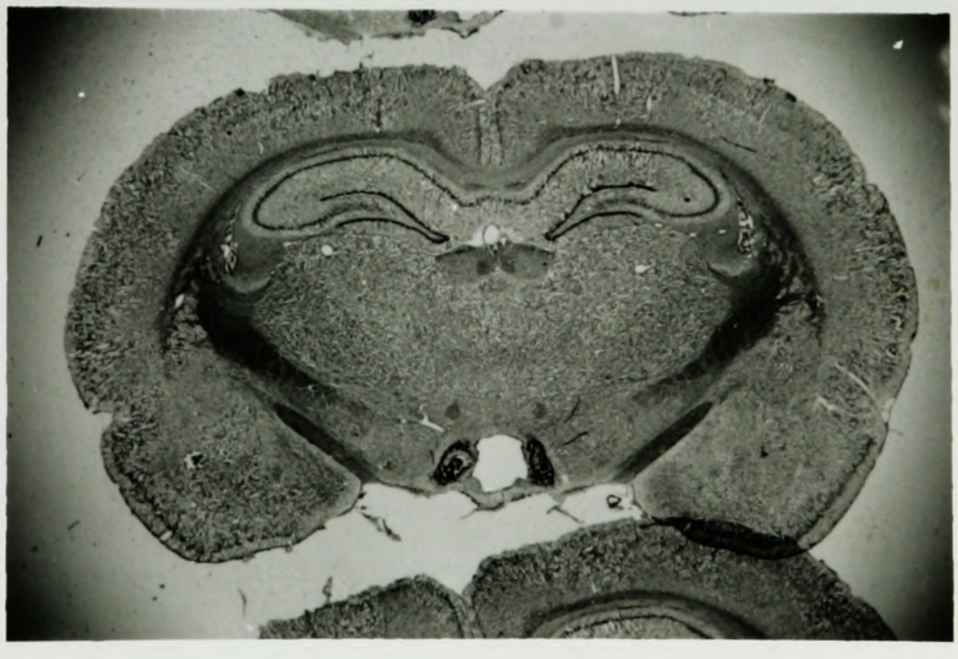
Electrolytic lesions of the VMH produced large ablations in this classically defined region. Overall, lesions were symmetrical, extended rostrally into the anterior hypothalamus reaching the posterior aspect of the paraventricular nucleus and extended caudally to the mamillary nucleus. In only one animal, lesions were comparatively small and were restricted rostrally just anterior to VMN and caudally just posterior to VMN but extended laterally beyond the nucleus. Lesions extended dorsally well into the dorsomedial hypothalamus ending approximately at the top of the third ventricle. Ventrally, they encompassed the ventral part of VMN but in all cases the base of the brain remained intact. Laterally, lesions extended to the fornix but none were observed to reach the lateral hypothalamic area. The fornix remained visible in all but two animals.

Range of Accepted Lesions:

Figure 1 is an enlarged photograph showing a coronal section from the largest and smallest VMH lesions accepted for data analysis. These, and all subsequent sections shown, lie at approximately the middle of the VMN in its anterior - posterior extent. Using Konig and Klippel's (1963) system, the center of VMN is at A4570. The smallest lesion accepted (lower photograph) was included because it fell within the VMN and appeared to remove the entire nucleus as well as some surrounding tissue. The largest lesion (upper photograph) clearly removed the nucleus and most of the DMH and parafornical hypothalamic areas. Although the fornix is not visible in this animal, the LHA and base of the brain are intact.

Figure 1. Largest (upper) and smallest (lower) lesions accepted for successful VMH lesions. Photographs are taken at the middle of VMN defined by reference to Konig and Klippel's atlas of the rat brain.





Parafornical Hypothalamic Knife Cut Lesions

Parafornical hypothalamic knife cuts successfully divided from the top of the third venticle to the base of the brain, the area between the fornix and VMN, bilaterally. Typically, knife cuts started anteriorly at the PVN and extended, caudally, to the lateral mammilary nucleus. In 1 animal, knife cuts extended anteriorly to the suprachiasmatic nucleus.

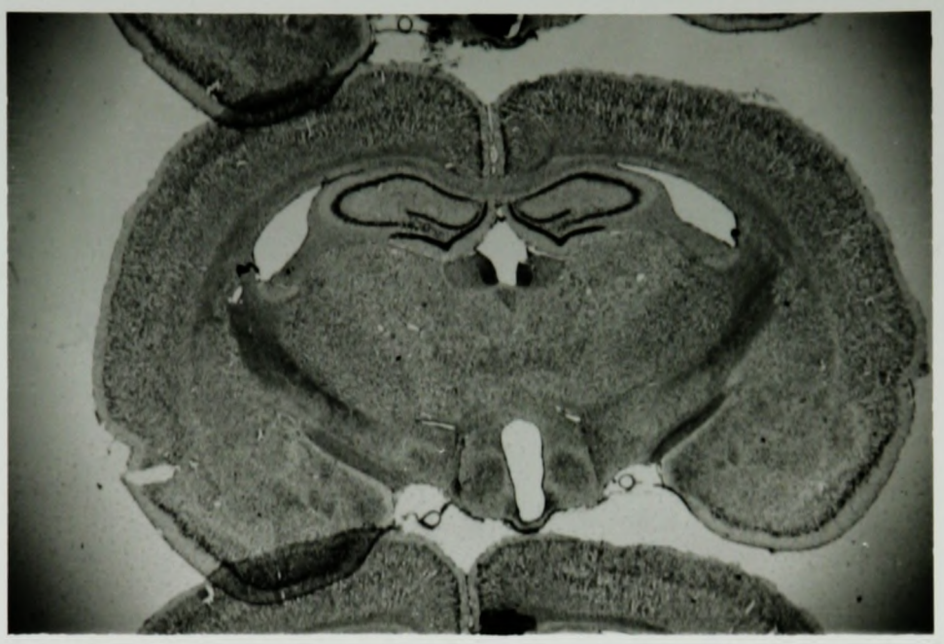
Overall, PFKC lesions were less symmetrical than VMH lesions. However, the width between cuts remained highly consistent. Therefore, when a cut encroached on VMN unilaterally, it encroached on the fornix contralaterally. In no case could bilateral damage to VMN, fornix, or LHA be detected. In two animals, the third ventricle was distended unilaterally possibly producing some unilateral damage to VMN or the dorsomedial hypothalamus (DMH).

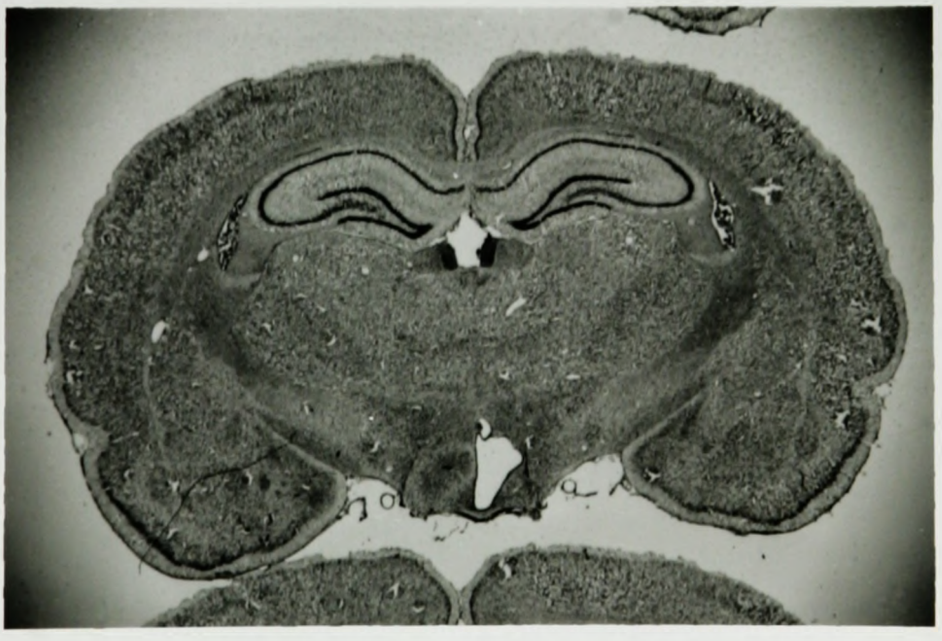
Range of Accepted Lesions:

Coronal sections of the best and worst placed knife cuts accepted for data analysis are shown in Figure 2. The best placed cuts (upper photograph) can be seen to excise an area between the fornix and VMN, from the top of the third ventricle to the base of the brain. These cuts are highly symmetrical and there appears to be no damage to VMN and only moderate dilation of the ventricle.

The lower photograph illustrates the poorest placed cuts accepted. There is substantial dilation of the ventricle causing nonspecific damage unilaterally. However, this animal was accepted because visible non-specific damage was restricted to one side of the brain, cuts extended along the entire length of the nucleus, and although the cut on the left

Figure 2. Best (upper) and worst (lower) placed knife cuts accepted for successful PFKC lesions.





side of the brain (right side of photograph) encroaches on VMN, it did not pass through the nucleus.

Ventromedial Hypothalamic Nucleus Lesions

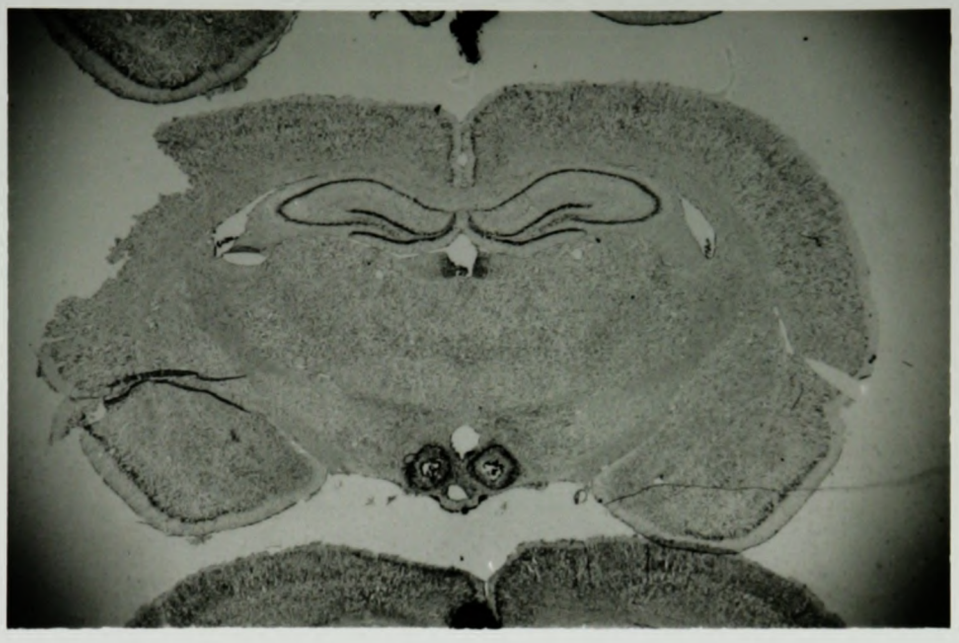
VMN lesions were highly symmetrical bilaterally and well centered in the nucleus in their medial-lateral orientation. Due to the length of the nucleus, it was not difficult to restrict lesions to VMN in their anterior-posterior orientation. In one rat, lesions extended rostral to VMN, producing slight damage to the anterior hypothalamus. The fornix and base of the brain remained observably intact in all brains. However, the ventricle was dilated in 3 rats and, therefore, some non-specific damage to the area surrounding VMN may have occurred in these animals.

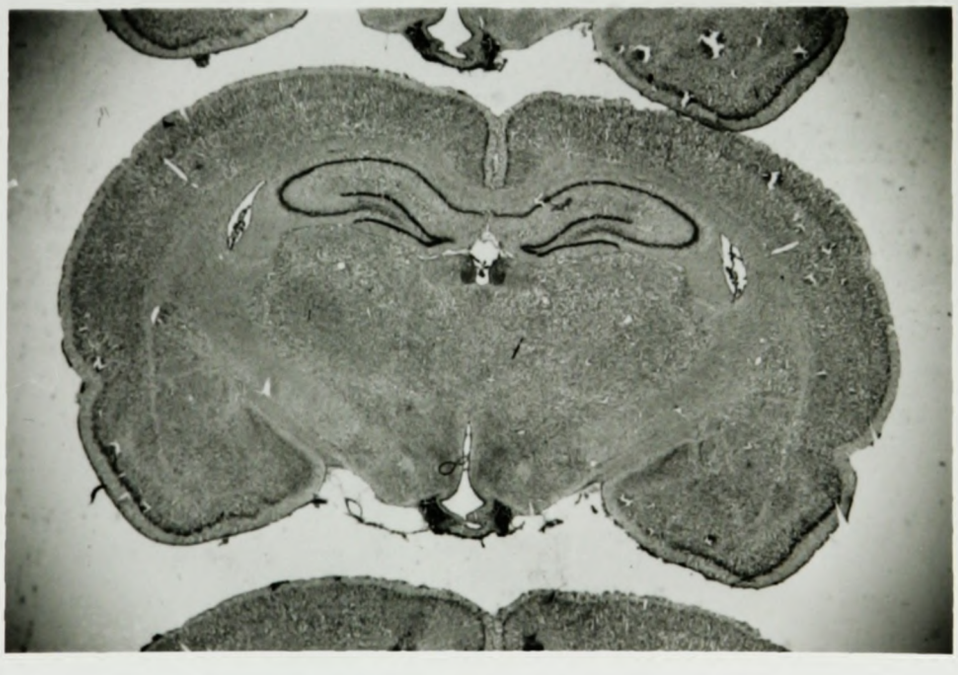
Range of Accepted Lesions:

The VMN lesion producing the most VMN damage, and consequently, the most non-specific damage is shown in Figure 3, upper photograph. The lesion in this animal, appears to consist of an obvious ablation that is centered in the nucleus, and a ring of necrosis around the perimeter of VMN. Although it is likely that cells within this entire region are destroyed, it is possible that functional cells remain between the hole and necrosis ring.

The poorest placed VMN lesion accepted is shown in the lower photograph. The lesion appears to be displaced ventrally. However, closer examination reveals the area below the ventricle to be compressed dorsally and therefore the lesion actually to lies within, rather than below, the nucleus. Comparison of the range of lesion damage in the VMN and VMH groups clearly indicates that distributions of lesion size are non-

Figure 3. Largest (upper) and smallest (lower) lesions accepted for successful VMN lesions.





overlapping between the two lesion conditions, with the largest VMN lesion being smaller than the smallest VMH lesion.

Caloric Intake

Group mean 24 hour caloric intakes over the 66 days of the experiment are presented in Figure 4. ANOVA was performed using subjects' mean intakes on each of the three diets in a 4 x 3 between/within design (i.e. Group x Diet). These data are displayed in Figure 5.

Overall, the groups differed in their level of caloric intake, as indicated by a significant effect for Group [$F(3,33) = 30.60$, $p < .001$]. The groups also differed as a function of the test diet, as indicated by a significant Group x Diet interaction, [$F(6,66) = 4.35$, $p < .001$]. Post-hoc analyses were performed to compare group mean caloric intakes on each diet and to examine changes in intake across diets within each group. F tests for simple effects performed at each level of diet found the three groups to be different in caloric intakes on Powder [$F(3,33) = 13.95$, $p < .001$], Mash [$F(3,33) = 18.39$, $p < .001$] and High Fat [$F(3,33) = 58.34$], $p < .001$]. Comparisons of lesion groups with SHAM rats produced the following findings. VMH rats consumed more calories than SHAM rats on Powder ($p < .01$), Mash ($p < .05$) and High Fat ($p < .01$). PFKC rats were also hyperphagic relative to SHAM controls on all three diets ($p < .01$ for all comparisons). VMN lesions produced no significant hyperphagia as indicated by an absence of any differences from SHAM rats on any of the diets.

Comparisons between the three lesion groups indicated the following. VMH rats consumed more calories per day than VMN rats on Powder and Mash ($p < .01$ for both comparisons) but these groups were not different on High Fat. PFKC rats had elevated caloric intakes relative to the VMN group on

Figure 4. Two-day group mean caloric intakes and body weights over the 66 days of Experiment 1a. Numbers in brackets are group sizes.

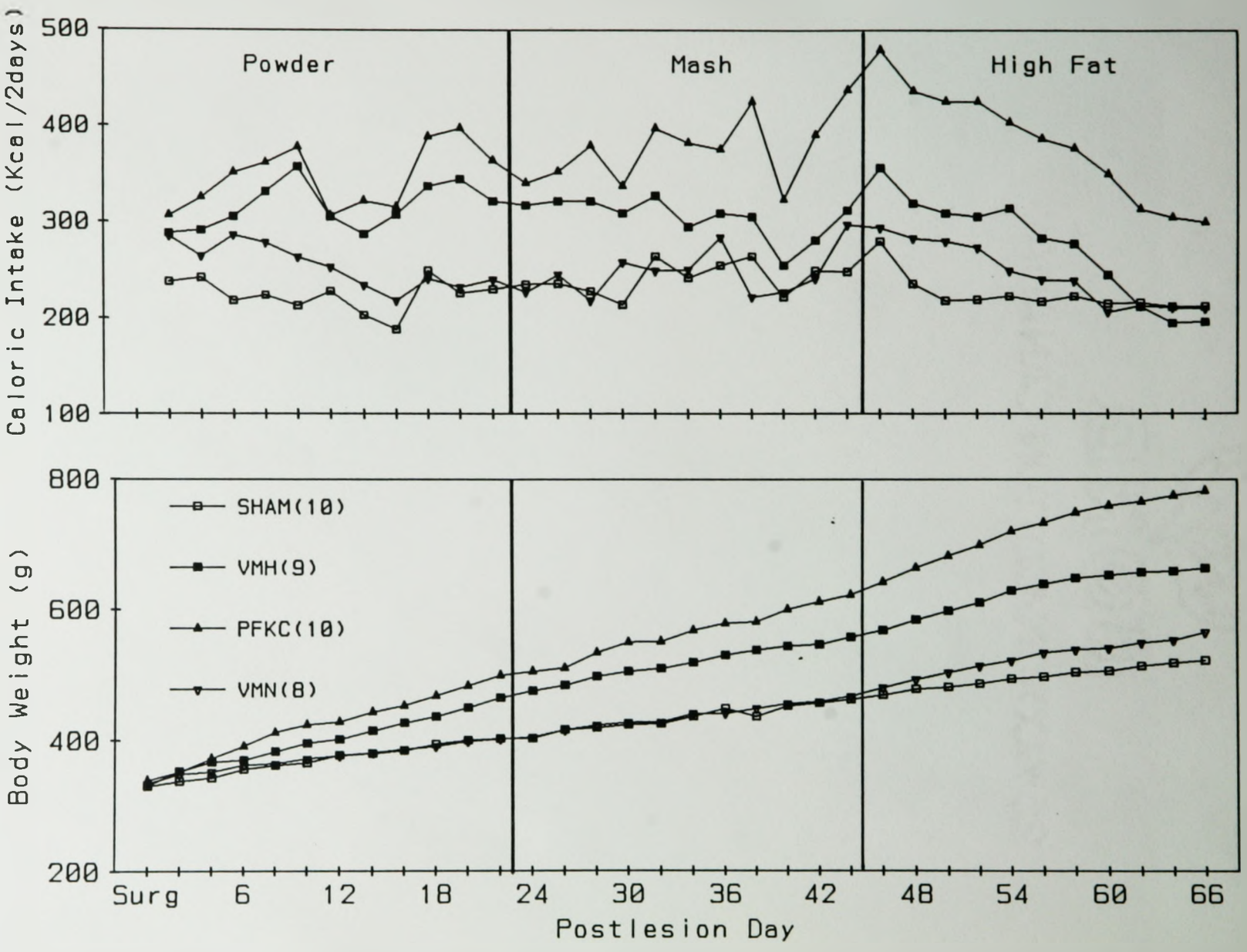
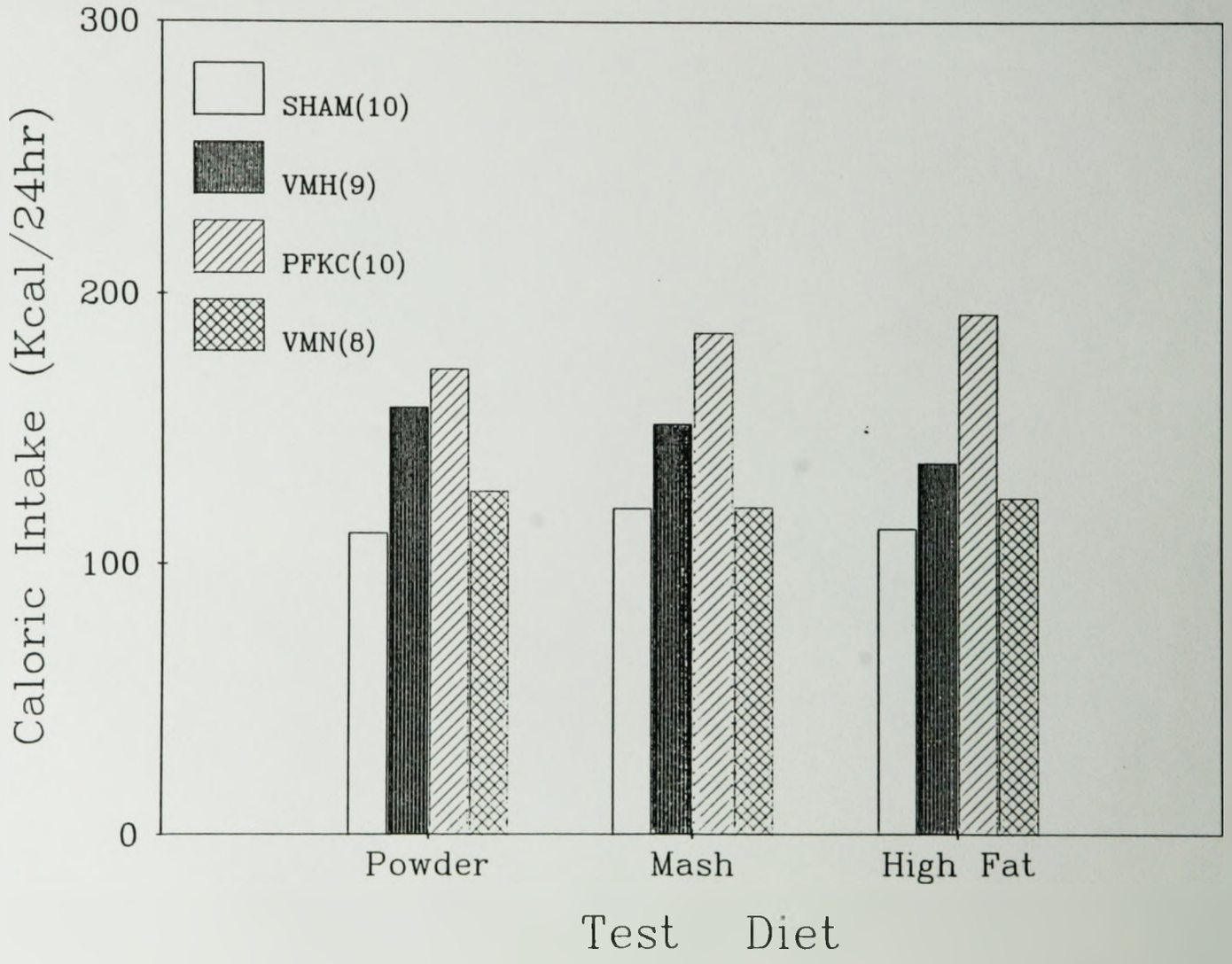

Caloric Intake (Kcal/2days)
500
400
300
200
100
Powder
Mash
High Fat
Body Weight (g)
800
600
400
200
SHAM(10)
VMH(9)
PFKC(10)
VMN(8)
Surg
6
12
18
24
30
36
42
48
54
60
66
Postlesion Day

Figure 5. Comparison of group mean 24 hour caloric intakes on three test diets.


300
200
100
0
SHAM(10)
VMH(9)
PFKC(10)
VMN(8)
Caloric Intake (Kcal/24hr)
Powder
Mash
High Fat
Test Diet

all three diets ($p < .01$ for all comparisons). Finally, the PFKC group also had elevated caloric intakes relative to VMH rats on Mash and High Fat ($p < .01$ for all comparisons) but were not different from the VMH group on Powder.

To examine the effects on intake of changing the test diet within lesion groups, multiple comparisons were performed using the Studentized Range statistic evaluated with the Newman Keuls procedure. This analysis produced the following findings. SHAM rats increased their caloric intake when their diet was changed from Powder to Mash and then decreased their intakes when Mash was replaced by the High Fat diet ($p < .05$ for both comparisons). Caloric consumption on High Fat was not different from the level of intake on Powder in SHAM rats. VMH rats showed no significant change in their level of intake on Mash relative to Powder but caloric intake on High Fat was reduced relative to both the Mash and Powder diets ($p < .01$ for both comparisons). PFKC rats showed a uniform increase in their level of intake across diets as indicated by elevated intake on Mash relative to Powder ($p < .01$) and increased consumption on High Fat relative to both Powder ($p < .01$) and Mash ($p < .05$). Finally, the VMN group showed no significant change in caloric intake as a function of test diet.

Body Weight

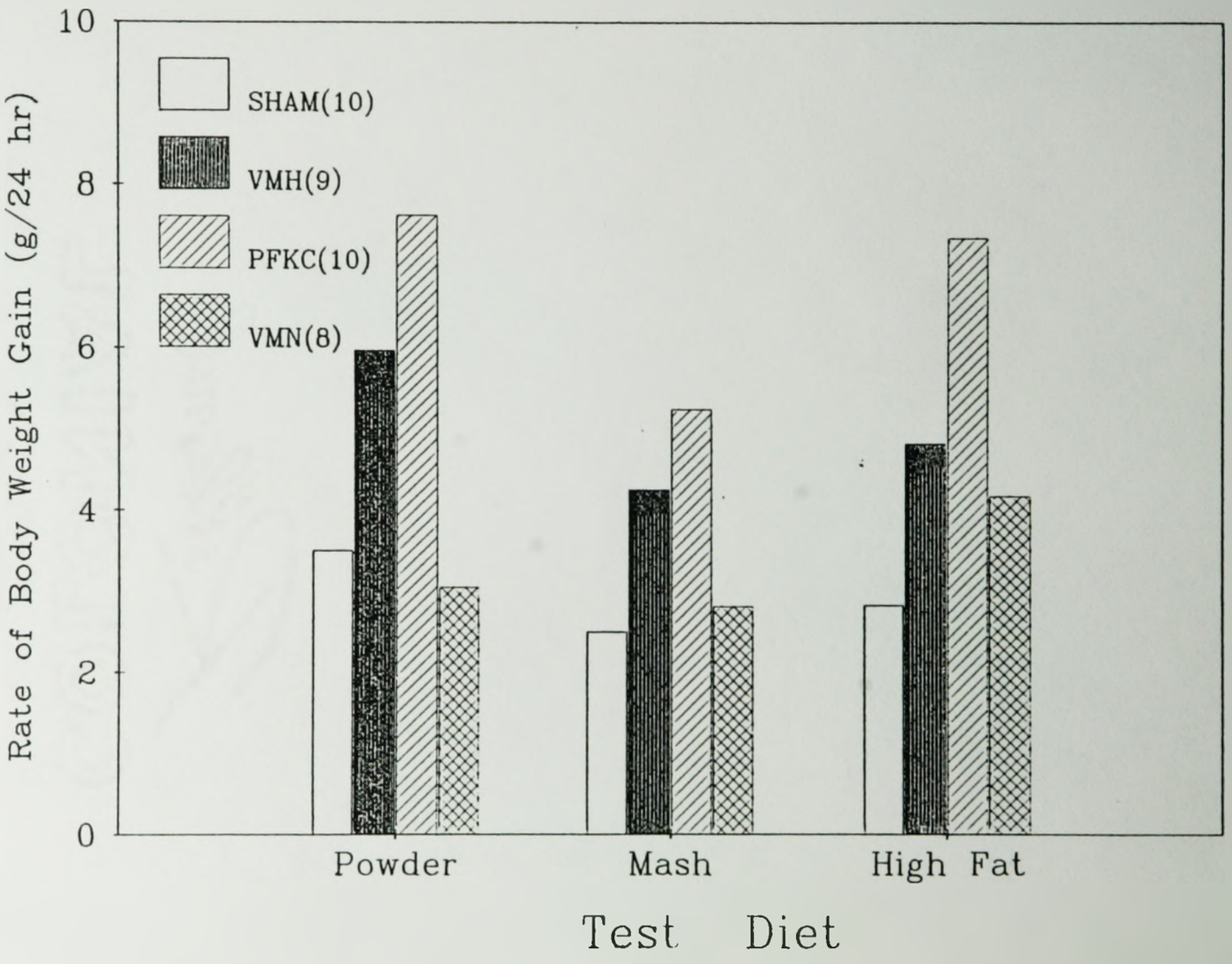
Group mean body weights, recorded every 2 days over the experiment, are presented in Figure 4. The first recording of body weight was made on the day prior to surgery (Surg). The groups were not different in weight at this time [$F(3,33) = 0.17$, N.S.]. However, comparison of group weights at surgery versus the final day of the experiment (Day 66) indicated that the groups gained weight at different rates producing a significant Group x

Time interaction [$F(3,33) = 37.46$, $p < .001$]. This effect was also demonstrated as a significant Group difference on Day 66 [$F(3,33) = 37.76$, $p < .001$]. Post hoc analyses indicated that both VMH and PFKC rats were heavier at the end of the experiment than SHAM and VMN rats ($p < .01$). Furthermore, PFKC rats were heavier than VMH rats ($p < .01$). VMN rats were not significantly heavier than SHAM rats.

To compare groups on the rate of weight gain on the 3 diets, daily weight gains were determined for each subject, and for each diet, by dividing the total weight gained on that diet by the number of days access to the diet. Group mean daily weight gains on each of the test diets are shown in Figure 6. ANOVA revealed a significant Group x Diet interaction indicating that group differences in weight gain were a function of maintenance diet [$F(6,66) = 2.27$, $p < .05$]. F tests for simple effects found the groups to be significantly different on Powder [$F(3,33) = 17.70$, $p < .001$], Mash [$F(3,33) = 11.35$, $p < .001$], and High Fat [$F(3,33) = 17.62$, $p < .001$]. Comparisons of each lesion condition with the SHAM group produced the following findings. VMH rats outgained SHAM rats on Powder ($p < .01$), Mash ($p < .01$), and High Fat ($p < .05$). PFKC rats also gained more weight per day that SHAM rats on Powder, Mash, and High Fat ($p < .01$ for all comparisons). The VMN group outgained SHAM rats on the High Fat diet ($p < .05$) but were not different from the control group on Powder or Mash.

Comparisons among VMH, PFKC, and VMN lesion groups found the following. The VMH group had higher daily weight gains than VMN rats on Powder ($p < .01$) and Mash ($p < .05$) but not on High Fat. PFKC rats outgained VMN rats on all three diets ($p < .01$ for all comparisons). PFKC rats gained more weight per day than VMH rats on Powder ($p < .05$) and High Fat ($p < .01$) but not on the Mash diet.

Figure 6. Comparison of group mean 24 hour weight gains on three test diets.


10
8
6
4
2
0
Rate of Body Weight Gain (g/24 hr)
SHAM(10)
VMH(9)
PFKC(10)
VMN(8)
Powder
Mash
High Fat
Test Diet

To examine further the effect of changes in diet on daily weight gains, post hoc multiple comparisons were used to compare mean weight gains between diets within each experimental group. In SHAM rats, rate of weight gain was reduced when their diet was changed from Powder to Mash ($p < .05$). When the diet was changed to High Fat, SHAM rats showed no significant change in weight gain, and their rate of weight gain remained reduced on High Fat relative to Powder ($p < .05$). A similar effect was seen in VMH rats. VMH rats had lower rates of weight gain on Mash and High Fat relative to the Powder diet ($p < .01$). There was no significant difference in weight changes between Mash and High Fat diets in the VMH group. PFKC rats, also demonstrated a reduction in weight gain when the diet was changed from Powder to Mash ($p < .01$). However, the high rate of weight gain observed on Powder was restored when the diet was changed to High Fat, as indicated by a significant increase in weight gain ($p < .01$) and an absence of any differences between weight gains on Powder and High Fat in this group. VMN rats, unlike the SHAM, VMH, and PFKC groups showed no significant reduction in weight gain when the Powder diet was changed to Mash. However, VMN rats increased their rate of weight gain on High Fat relative to both Mash and Powder diets ($p < .01$ for both comparisons).

Feed Efficiency

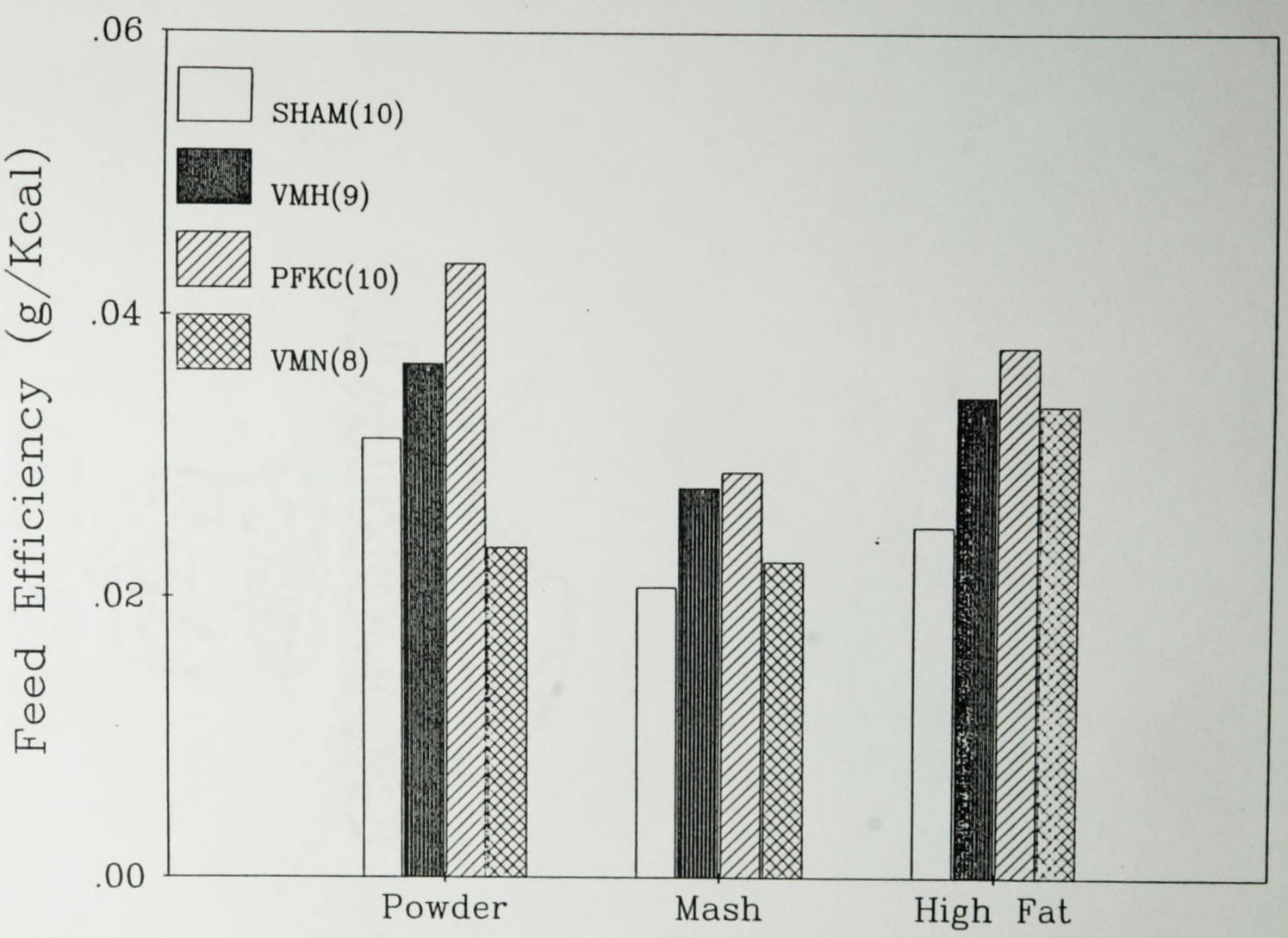
An index of feed efficiency was determined in each animal, defined as the ratio of weight gained to calories eaten (i.e. Efficiency = weight gain/ calories consumed). Feed efficiency was calculated for the entire experiment using the 66 day weight gains and cumulative caloric intakes, and for each diet using the 22 day weight gains and cumulative caloric intakes for each animal. Group mean feed efficiency scores on each diet

are shown in Figure 7. The groups were significantly different in efficiency scores over the entire experiment, [$F(3,33) = 7.68$, $p < .001$]. Multiple comparisons demonstrated that VMH rats gained more weight per calorie consumed than SHAM or VMN rats ($p < .05$). PFKC rats also were more efficient than the SHAM and VMN groups ( $p < .01$ for both comparisons). There were no other significant group differences.

The groups differed in feed efficiency as a function of diet, $F(6,66) = 3.17$, $p < .01$. To locate the source of this interaction, group mean efficiency scores were compared within each level of diet, and between diets within each lesion group. The groups differed in feed efficiency on Powder, [$F(3,33) = 14.37$, $p < .001$], Mash [$F(3,33) = 4.67$, $p < .01$], and High Fat [$F(3,33) = 4.14$, $p < .01$]. VMH rats gained more weight per calorie eaten than the SHAM rats on Mash and High Fat ($p < .05$ for both comparisons) but did not differ from the SHAM group on Powder. However, PFKC rats had higher efficiency scores than SHAM rats on all three test diets ($p < .01$ for all comparisons). VMH and PFKC rats were more efficient than VMN rats on Powder ($p < .05$) but VMH and PFKC groups did not exceed VMN rats on the Mash and High Fat diets. PFKC rats were more efficient than VMH rats on Powder only ($p < .05$).

A major contribution to the Group x Diet interaction was caused by the dramatic change in feeding efficiency of VMN rats across diets. VMN rats gained less weight per calorie than SHAM rats on Powder ($p < .05$), were no different from SHAM rats on Mash, but were more efficient on the High Fat diet ($p < .05$).

Figure 7. Comparison of group mean feed efficiency scores on three test diets.


.06
.04
.02
.00
Feed Efficiency (g/Kcal)
SHAM(10)
VMH(9)
PFKC(10)
VMN(8)
Powder
Mash
High Fat
Test Diet

Obesity

Carcass composition data are presented in Table 2. The groups differed in percentage carcass fat [$F(3,33) = 80.28$, $p < .001$]. Multiple comparisons indicated that VMH, PFKC and VMN lesions all produced increased percentage fat relative to SHAM lesions ($p < .01$ for all comparisons). Rats with VMH lesions had a greater percentage fat than rats with either VMN ($p < .01$) or PFKC lesions ($p < .05$) alone. PFKC rats were more obese than the VMN group ($p < .05$).

Table 2. Group Mean Carcass Composition Measures For Rats Maintained on Ad Libitum Feeding.

| GROUP | CARCASS WEIGHT | PERCENT WATER | PERCENT FAT |
|---|---|---|---|
| SHAM (10) | 440.0 (13.2) | 57.5 (0.8) | 22.9 (1.0) |
| VMH (9) | 579.9 (21.7) | 34.1 (1.5) | 52.6 (1.9) |
| PFKC (10) | 682.2 (12.6) | 37.7 (1.1) | 48.1 (1.3) |
| VMN (8) | 489.8 (21.3) | 42.0 (1.2) | 42.6 (1.5) |

Numbers in parentheses are 1 standard error of the mean.

Anatomical Substrate of Hyperphagia

Since the brain co-ordinates of PFKC lesions are chosen to interrupt a system involved in hypothalamic hyperphagia, the anatomical locus of knife cuts should affect caloric intake. Figure 8 compares the individual

average caloric intakes over the 66 day experimental period by animals with misplaced PFKC lesions against successful PFKC lesions and SHAM groups. One animal had extensive nonspecific damage and therefore was excluded from the analysis. The first three subjects (27, 37, 42) had cuts that were too posterior, starting just caudal to the anterior aspect of VMN. Subjects 2,39, and 56 had lesions that were well placed anteriorly but misplaced laterally so that the knife passed through VMN unilaterally and encroached on the LHA contralaterally.

It can be seen that overall, the caloric intakes were less than the mean for animals with well placed knife cuts indicating that such lesions were generally less effective for producing hyperphagia. A t test comparing mean intakes for PFKC rats having anatomically successful lesions ($\bar{X}$ = 184 Kcal/day) with rats having misplaced knife cut lesions ($\bar{X}$ = 168 Kcal/day) was statistically significant, [$t(14) = 2.67$, $p < .05$].

The small lesions used to ablate the VMN might produce hyperphagia if they were in a position to disturb the hypothalamic feeding system. According to the model of the feeding inhibitory system suggested by Sclafani and Berner (1977, Figure 12 of their publication) small lesions falling lateral to VMN or just rostral to the nucleus might produce overeating. VMN lesions in the present study rarely fell lateral to VMN. However, since VMN lesions tended to vary on their anterior - posterior location, some lesions fell rostral to the nucleus, in the area suggested by Sclafani and Berner (1977) as the origin (or destination) of the feeding inhibitory fibres. Therefore, the relationship between the locus of VMN damage and daily caloric intake was examined by ranking lesions on their anterior-posterior locus. Figure 9 displays the average 66 day caloric intakes in ad libitum fed rats ranked by the rostral boundary of the lesion

Figure 8. Comparison of individual mean 24 hour caloric intakes for subjects with misplaced PFKC lesions, with the mean intake of accepted PFKC lesion rats and SHAM rats.

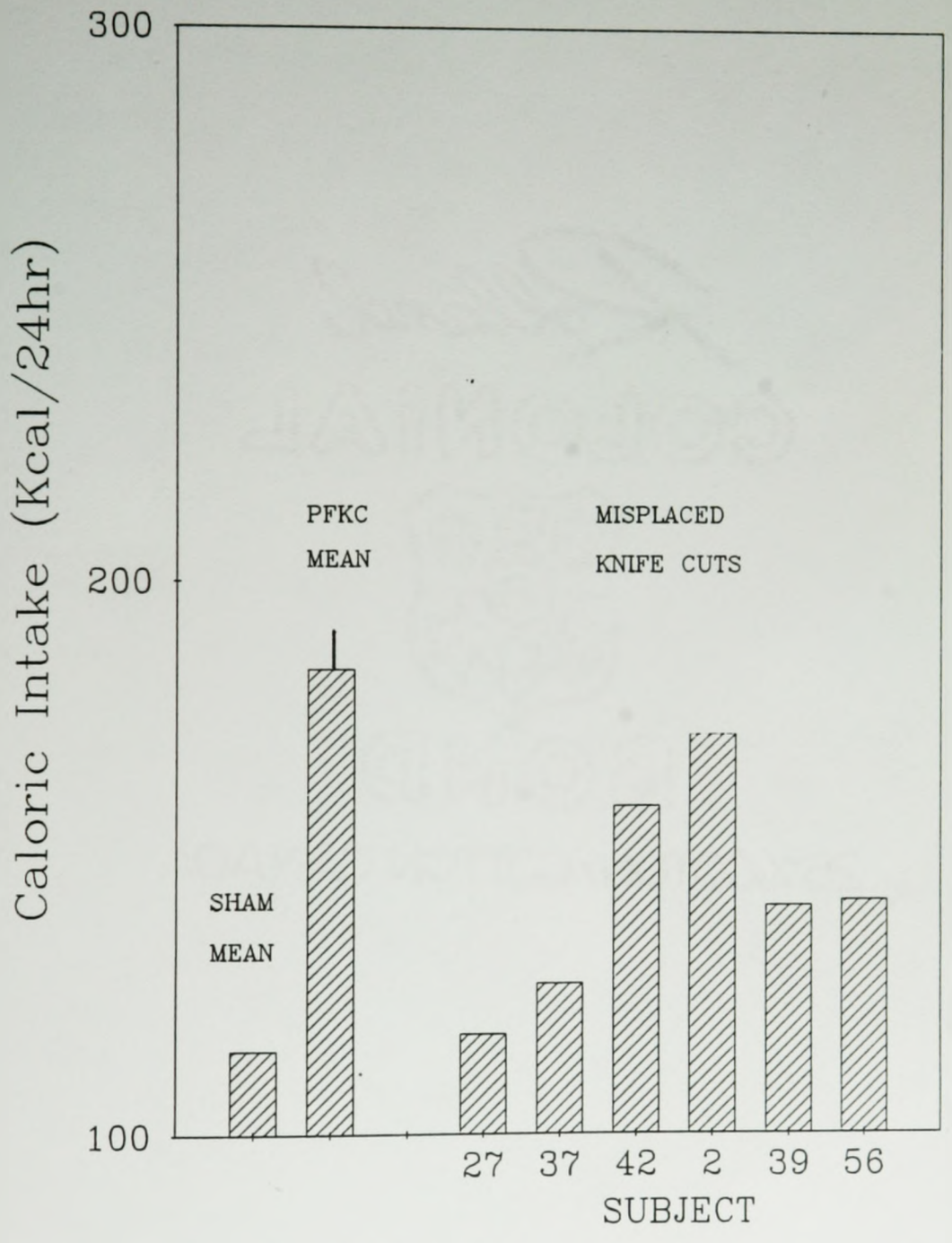
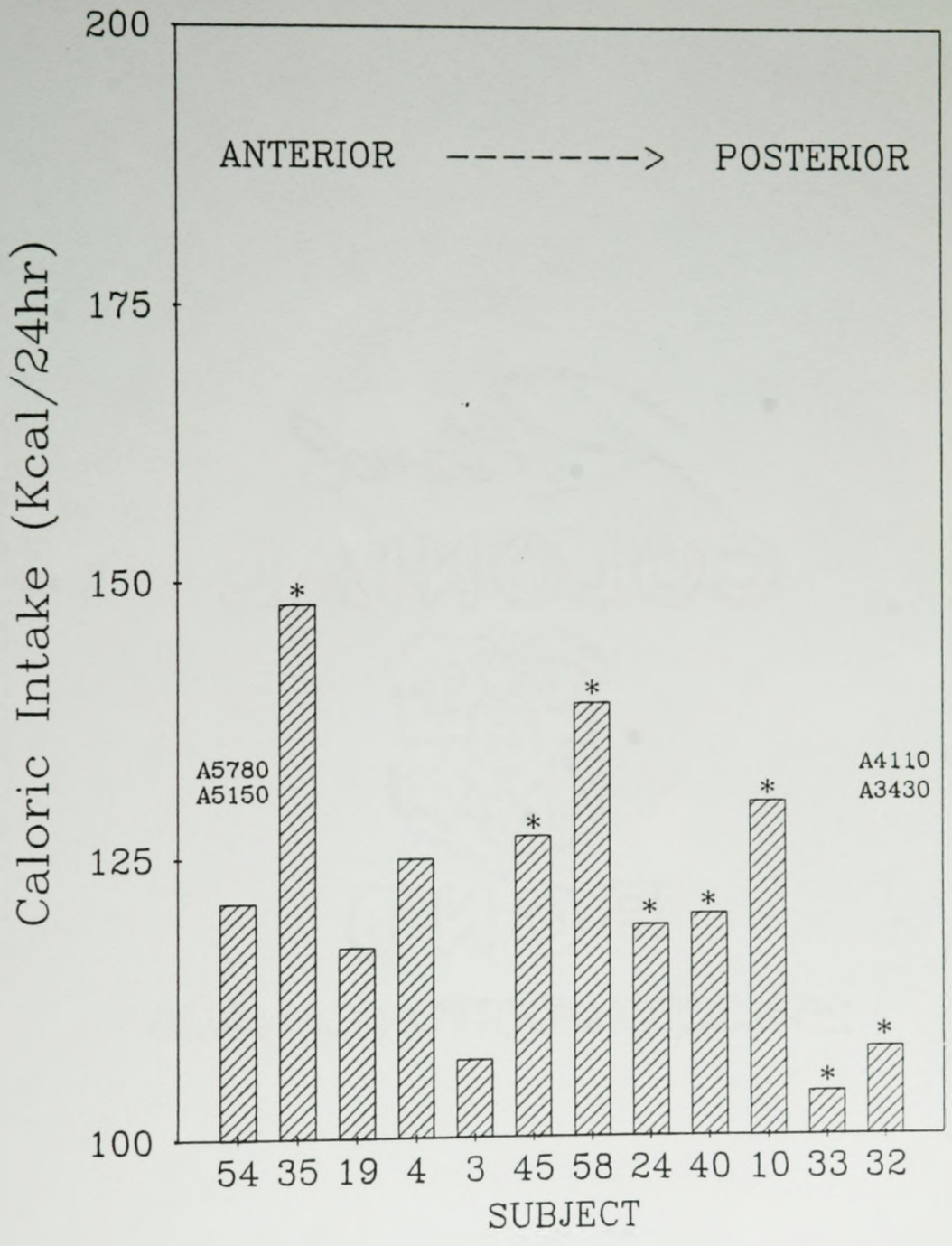

300
200
100
Caloric Intake (Kcal/24hr)
PFKC
MEAN
MISPLACED
KNIFE CUTS
SHAM
MEAN
27
37
42
2
39
56
SUBJECT

Figure 9. Individual mean 24 hour caloric intakes for VMN lesion rats (accepted and not accepted) ranked according to the anterior border of the lesion. Numbers above the most rostral and most caudal lesions are the anterior-posterior range of damage based on coordinates in Konig and Klippel's (1963) atlas of the rat brain. Asterisks indicate animals accepted as successful lesions.


200
175
150
125
100
Caloric Intake (Kcal/24hr)
ANTERIOR -------> POSTERIOR
A5780
A5150
A4110
A3430
*
54 35 19 4 3 45 58 24 40 10 33 32
SUBJECT

(parallel ranking of the caudal boundary would be expected given similar sized lesions). Contrary to the prediction, there was no overall relationship between anterior - posterior locus of the lesion and caloric intake, as indicated by a non-significant Spearman rank order correlation, rho = 0.35 [t(10) = 1.18, N.S]. Furthermore, a t test comparing mean caloric intakes between rats with lesions that fell inside VMN (subjects 45 - 32 in Figure 9) with rats having lesions anterior to the nucleus (subjects 54, 35, 19, 4, 3) was not significant [t(10) = 0.054, N.S.].

## DISCUSSION

The major findings of this experiment are summarized below. The essential findings are depicted in Table 3.

(1) VMN lesions did not produce hyperphagia on any of the three test diets used;

(2) VMN lesions produced obesity, as defined by a greater percentage body fat relative to SHAM operated control rats;

(3) PFKC lesions produced an increase in percentage carcass fat (i.e. obesity) when permitted to eat ad libitum;

(4) PFKC lesions were as effective as VMH lesions in producing hyperphagia and weight gain. In fact, PFKC rats were more hyperphagic than VMH rats on the Mash and High Fat diets. This group difference lead to an overall increase in daily caloric intakes and final weight gain by PFKC rats relative to the VMH group. However;

(5) Carcass analysis indicated that VMH lesions produced significantly greater obesity than PFKC lesions.

(6) VMH, PFKC, and VMN lesions differed in their effects on feed efficiency. VMN lesions increased the rate of weight gain per calories consumed on High Fat only. VMH lesions increased feed efficiency on Mash and High Fat. PFKC lesions increased feed efficiency on all three test diets.

Table 3. Results of Experiment 1a.

| GROUP | CALORIC INTAKE | BODY WEIGHT | %FAT |
|---|---|---|---|
| VMH | + | + | +++ |
| PFKC | ++ | ++ | ++ |
| VMN | - | - | + |

Symbols represent significant differences from control rats. Plus indicates an increase. Dash indicates no change. Double and triple plus indicates significantly elevated relative to other lesion groups.

The contribution made to research on hypothalamic obesity by these observations, and their relevance to a dissociative perspective of the VMH syndrome, are discussed in turn.

The failure of lesions of the ventromedial hypothalamic nucleus to produce marked overeating and weight gain has been previously documented (Gold, 1973). Gold found that small lesions restricted to the VMN produced no detectable overeating relative to control animals, even on a relatively palatable high fat diet. The diet used by Gold consisted of Purina Powder and Crisco Oil mixed in the same ratio as the present experiment (i.e. 67% powder, 33% oil) but melted shortening was used in that study rather than liquid oil (present study). Therefore, both studies demonstrate the failure of VMN lesions to produce hyperphagia on a palatable diet. The present experiment provides a better documentation of this effect by demonstrating the absence of hyperphagia on a variety of test diets. Therefore, these findings provide stronger support for one hypothesis congenial with a dissociative perspective of the VMH syndrome; namely, that the VMN is not involved in hypothalamic hyperphagia.

One noteworthy difference between the present findings and those of Gold (1973) was the significant elevation in daily weight gain observed in VMN rats on the High Fat diet. Gold reported an absence of significant weight gains in VMN rats that were maintained on a similar diet. It is unlikely that this difference between studies is accounted for by the size of the lesions. The VMN lesions in the present study were slightly smaller than those used by Gold, who employed lesion parameters that resulted in a power of 10 mcoulomb. The VMN lesions in the present study were 8 mcoulomb (1.0 mA x 8 sec.). A second possibility is that differences in the exact locus of VMN lesions between these studies may account for this inconsistency. Gold did report that more ventral VMN lesions produced a small but significant weight gain. It is possible that the small increase

in body weight on High Fat seen in VMN rats in the present study resulted from damage to the area just ventral to the VMN.

This is the first experiment documenting levels of body fat following electrolytic lesions restricted to the VMN in mature animals. VMN lesions produced an elevation in percentage body fat. Since VMN rats demonstrated no overall increase in caloric intake or body weight relative to the control group, this finding supports the hypothesis that damage to VMN accounts for the hyperphagia-independent obesity observed following VMH lesions (i.e. metabolic obesity). Furthermore, this finding strongly suggests that the hypothalamic mechanism(s) involved in metabolic obesity in mature rats are anatomically distinct from the substrate(s) involved in feeding.

There is an alternative explanation of the present findings on VMN rats. A significant metabolic obesity in the absence of significant hyperphagia may be a function of differences in measurement sensitivity between the two variables. Thus, the obesity found in VMN rats may result from relatively small levels of overeating that were not detected. The fact that PFKC rats are grossly hyperphagic relative to VMN rats, but are only slightly more obese, suggests that this possibility is unlikely. However, this alternative is addressed directly in Experiment 2.

The present study provides the first demonstration of an increased percentage body fat following PFKC rats as determined by carcass composition. Measurements of body composition replicate the well established obesity produced by VMH lesions. However, until the present experiment, no evidence has established the existence of obesity in PFKC rats. This experiment indicates that the overeating produced by PFKC

lesions leads to both excessive weight gain, and increased percentage body fat.

PFKC lesions were more effective than VMH lesions for producing hyperphagia and weight gain. A similar effect has been reported by Sclafani and Aravich (1983) who found that PFKC lesions produced a significantly greater weight gain but not significantly greater caloric intake than VMH lesions. However, the latter researchers determined caloric intakes at three points in time over the 60 days of their experiment. Comparisons of the daily caloric intakes of VMH and PFKC rats were not made between days 15 and 55 in that study. Visual examination of 5 day total caloric intakes over this period (Figure 2 in their study) indicates that PFKC rats exceeded VMH rats over this period. Therefore, it is possible that more detailed statistical analyses of their data would reveal that PFKC lesions are more effective for producing hyperphagia than VMH lesions, at least over a limited period of time postlesion. The more detailed comparisons between VMH and PFKC lesions of the present study provide an advantage over that study and could account for this discrepancy.

Two possible explanations of the greater effect on feeding behaviour of PFKC lesions are: (1) VMH lesions are not large enough or accurately placed to produce the maximum hyperphagia and weight gain or; (2) additional damage to other mechanisms by VMH lesions inhibits the effects of the lesion on caloric intake. This issue is addressed in Experiment 1b.

In addition to replicating previous observations of a quantitative difference between VMH and PFKC lesions on caloric intake, comparisons made between caloric intakes of lesion groups as a function test diets also suggest that qualitative differences in feeding abnormalities may exist

between these two ablation techniques. PFKC rats were more hyperphagic than VMH rats throughout the middle and latter parts of the experiment when maintained on Mash and High Fat respectively. However, the groups did not differ initially, when maintained on Powder. It is, of course, not possible to conclude that the relatively greater hyperphagia in PFKC rats is caused by an enhanced effect of diet palatability on caloric intake in this group, because determination of the effects of diet on caloric intake are confounded by the introduction of diets at different times postlesion. Therefore, the relatively greater hyperphagia of the PFKC group relative to the VMH group on High Fat may be caused by a delay in the onset of dynamic and static phases of the syndrome following PFKC lesions. In fact, examination of Figure 4 suggests that caloric intake returns to near normal levels (i.e. the static phase) in VMH rats sooner than in PFKC rats.

Since determination of body composition following PFKC lesions has not been performed previously, the finding that VMH lesions produced a significantly greater obesity than PFKC lesions is also a novel observation. Since PFKC rats were more hyperphagic overall than VMH rats, this finding also suggests that VMH lesions were relatively more effective for producing metabolic changes favouring fat accumulation than PFKC lesions. This finding is consistent with the hypothesis that PFKC lesions are less effective than VMH lesions for producing metabolic obesity, and therefore, supports a dissociative perspective of the VMH syndrome. More conclusive evidence for differences between VMH and PFKC lesions on metabolic mechanisms is provided by Experiment 2.

Measurements of feed efficiency in the present study support the previous findings of Sclafani and Aravich (1983) that both VMH and PFKC lesions increase the ratio of weight gained to calories consumed. The

present study further demonstrates that increases in feed efficiency in both VMH and PFKC groups were maintained over three test diets. No previous research has examined the effects of VMN lesions on feed efficiency. In the present experiment, VMN rats were found to have elevated efficiency scores on the High Fat diet only.

One difference between the present findings and those of Sclafani and Aravich (1983) was that the latter researchers observed that efficiency scores were significantly increased in PFKC rats relative to VMH rats. In contrast, there was no overall difference between VMH and PFKC rats on feed efficiency in the present experiment. An explanation for this discrepancy is not apparant. One major difference between the two studies is that the animals in the Sclafani and Aravich experiment were maintained simultaneously, on three diets rich in either carbohydrate, protein, or fat. This permitted animals to control the proportions of the three macronutrients that made up their diet. It is possible that this regimen permitted PFKC rats to display a relatively greater feed efficiency than the present procedure.

Feed efficiency is a relatively recent measure in the literature on hypothalamic obesity. It is not yet understood whether changes in efficiency reflect a direct lesion-induced change in physiological mechanisms, or whether feed efficiency is simply a consequence of changes in caloric intake or weight gain. In support of the latter view, increases in feed efficiency in the present experiment paralleled increases in intake and body weight. Efficiency scores increased in VMH and PFKC groups on all diets that supported excessive weight gain. Similarly, efficiency scores were elevated in VMN rats only on the High Fat diet, which also produced excessive weight gain in this group. Finally, Sclafani and Aravich (1983)

found that feed efficiency scores were similar between rats matched for weight gain following PFKC or VMH lesions.

Changes in body weight have been commonly used as an index of obesity in animal studies. However, observations made in this experiment indicate that changes in body weight are not always correlated with changes in body fat. In fact, precise definition of obesity, i.e. an elevated percentage body fat, can lead to quite different conclusions about the effects of hypothalamic lesions on obesity, compared to measurement of weight only. For example, VMN lesions would not be concluded to produce obesity if body weight were used as the index of fat accumulation. This fact has led some researchers to discount the VMN as being important in the VMH obesity syndrome (e.g. see LeMagnen, 1983; Gold, 1973). When percentage carcass fat is measured, VMN lesions are found to clearly increase adiposity and therefore, damage to VMN is relevant to the obesity syndrome. The importance of this distinction is highlighted further by another observation. VMH lesions were found to produce a greater obesity than PFKC lesions, when defined as percentage carcass fat, but PFKC lesions produced greater obesity when defined in terms of body weight gain.

Examination of the relationship between the locus of knife cuts and caloric intake produced evidence in support of an existing model of hypothalamic hyperphagia (Sclafani & Berner, 1977; Sclafani & Kirchgessner, 1986). It was originally thought that VMN was the relevant site for producing hyperphagia by VMH lesions. However, this view is contradicted both by the failure of VMN lesions to produce hyperphagia, and by the effectiveness of PFKC lesions for producing overeating. Currently, hypothalamic hyperphagia is considered to be due to the interruption of a longitudinal fibre system connecting hypothalamic nucleii with lower brain

stem centers. More specifically, from caudal to rostral, this fibre system is believed to extend from the lower brain stem, possibly the nucleus of the solitary tract, pass just lateral to VMN and turn medially rostral to VMN, to terminate or originate at, or just posterior to, the paraventricular nucleus (PVN). This model assumes that PFKC lesions are effective because they sever fibres as they turn medially rostral to VMN. Therefore, cuts should be less effective if placed more laterally or posteriorly. Second, while small lesions in VMN do not produce hyperphagia, they might affect eating if placed rostral to VMN, between VMN and PVN. The first prediction was confirmed, here, by comparison of caloric intakes between successful and misplaced PFKC lesions. The second prediction was not confirmed. This finding does not disprove the hypothesis. The anterior VMN lesions in this experiment were small, and the exact locus of the hypothetical fibre system has not been specified. Therefore, the anterior VMN lesions may not have been sufficiently placed to interrupt this fibre system.

In summary, this experiment produced the following findings that support a dissociative perspective of the VMH syndrome. First, VMH and PFKC lesions produce hyperphagia and weight gain but VMN lesions do not. Second, VMN lesions produce obesity in the absence of hyperphagia. Third, VMH lesions are more effective than PFKC lesions for increasing percentage body fat. However, knife cuts were more effective than VMH lesions for producing overeating and weight gain. This difference does not contradict a dissociative perspective of the VMH syndrome, but the dissociative model does not explain this group difference. One possible explanation of the greater hyperphagia effect of PFKC lesions is relevant to the dissociative perspective. Specifically, it is possible that damage to VMN actually

## EXPERIMENT 1B

The results of Experiment 1a support the hypothesis that PFKC lesions produce the hyperphagia and weight gain characteristic of classic VMH lesions. However, PFKC rats were more hyperphagic and gained more weight than VMH rats. The most salient difference between VMH lesions and PFKC lesions is that VMH lesions include damage to VMN but PFKC lesions do not. Therefore, destruction of VMN may actually limit the degree of overeating and weight gain produced by medial hypothalamic damage. To test this hypothesis, the present experiment examines the effects on caloric intake and body weight gain of combined PFKC and VMN lesions. If the explanation is correct, VMN damage should reduce the effectiveness of PFKC lesions for producing hyperphagia and weight gain. More generally, this experiment increases the comprehensiveness of the analysis by determining the effects of combined PFKC and VMN lesions on food intake and body weight changes.

### METHODS

Fifty-six rats were assigned to the following five experimental conditions such that groups were similar in mean weight and variance at the time of surgery; Ventromedial Hypothalamic lesions (VMH), Parafornical Hypothalamic Knife Cut lesions (PFKC), Combination PFKC and VMN lesions (PFKC/VMN), Sham lesions (SHAM-L/SHAM-KC), and a no-lesion control

group (CONT). Lesions for VMH and PFKC groups were the same as in Experiment 1a. For group PFKC/VMN, rats received VMN lesions as defined in General Methods, followed immediately by PFKC lesions.

Two control groups were used. SHAM-L/SHAM-KC rats underwent combined VMH and PFKC sham surgeries. Therefore, rats in this group were anesthetized, four skull holes were made, and the electrode and knife cannula were lowered but no current was passed, and the knife was not extended or lowered through VMH. CONT rats received no surgery. Thus, the following groups were formed: VMH (n=12); PFKC (n=16); PFKC/VMN (n=18); SHAM (n=5); CONT (n=5). The ultimate group numbers for statistical analysis, based on histological findings were: VMH (n=6); PFKC (n=10); PFKC/VMN (n=7); SHAM-L/SHAM-KC (n=5); CONT (n=5).

All rats were maintained on the Mash test diet starting 24 hours following surgery and continuing for 60 days. Caloric intake and body weight were monitored every second day as in Experiment 1a. On day 60, food cups were removed and rats were sacrificed 24 hours later. Brains were removed for histological analyses as described in General Methods.

## RESULTS

### Histological Findings

The criteria used to include subjects in each brain lesion condition for data analysis were described in General Methods, and the range of accepted VMH and PFKC lesions was described in Experiment 1a. These criteria were maintained, and all subjects accepted for data analysis fell within the ranges shown in Experiment 1a. Therefore, only histological findings for the PFKC/VMN group are described here.

Combined PFKC/VMN lesions produced large ablations in the VMH. These lesions resembled relatively large electrolytic VMH lesions in that they tended to produce substantial dilation of the third ventricle and consequently, likely produced non-specific damage to DMH and the anterior and posterior hypothalamus. As in Experiment 1a, both knife cuts and VMN lesions tended to vary in their anterior-posterior placement. However, in all cases, knife cuts passed laterally to the entire extent of VMN, and VMN lesions were observed to lie within the nucleus.

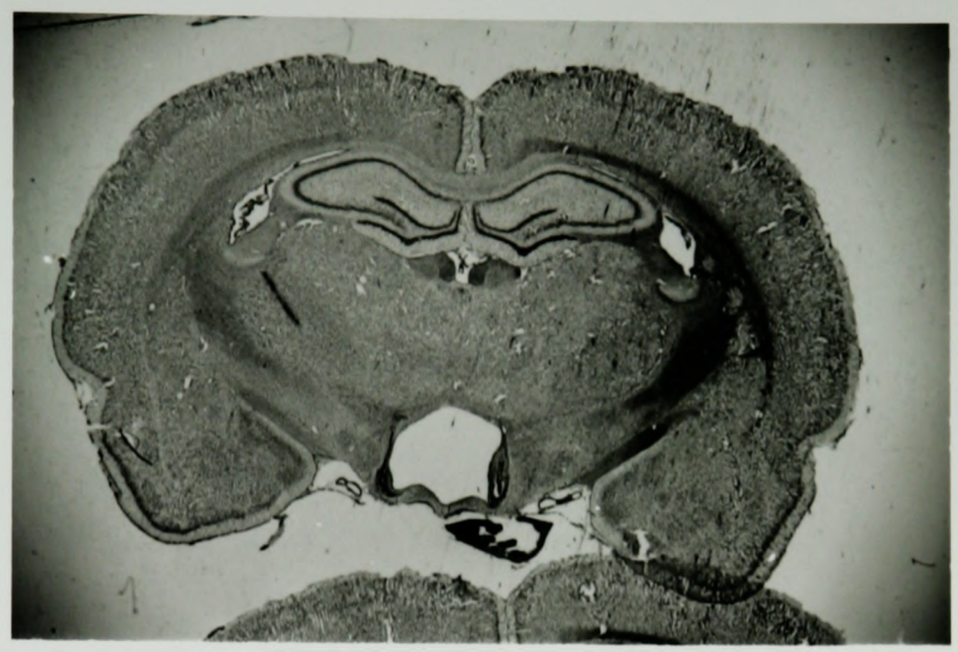
Knife cuts started at the rostral aspect of PVN in 2 rats, at the caudal PVN in 3 rats, and midway through PVN in the remaining 2 animals. VMN lesions began at the anterior border of the nucleus in 2 rats, and were well centered in the nucleus in the remaining 5 rats. In all but 1 animal, the ventricle was considerably dilated. In the one exception, there was a marked, bilateral, loss of tissue around the knife cuts.

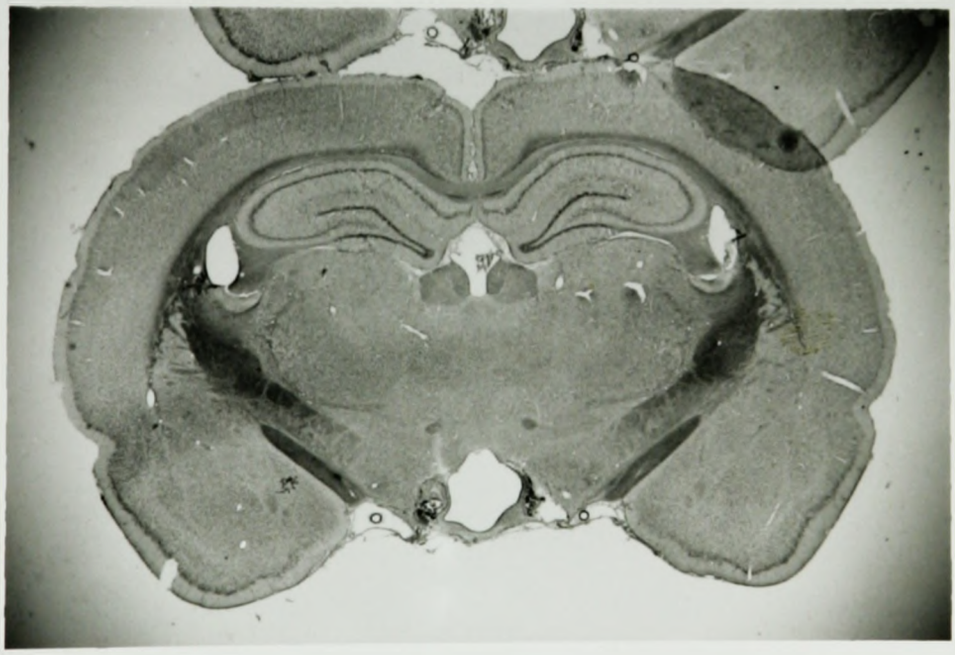
Range of Accepted Lesions:

Figure 10 shows enlarged photographs of the largest (upper) and smallest (lower) PFKC/VMN lesions included in data analysis. Photographs show coronal slices at approximately the center of VMN. In the upper photograph, both knife cuts and VMN lesions are clearly visible and are highly symmetrical. Knife cuts extend from the top of the third ventricle to the base of the brain. Lesions are well centered in the nucleus. The large dilation of the third ventrical is typical of animals in this group.

Lesions in the lower photograph are considered to be smaller than those in the upper photograph because knife cuts do not appear to extend to the top of the ventricle, the ventricle is less dilated, and the VMN lesion on the left side of the brain may miss part of the ventromedial aspect of

Figure 10. Best (upper) and worst (lower) placed combined knife cut/ electrolytic VMN lesions accepted for successful PFKC/VMN lesions.





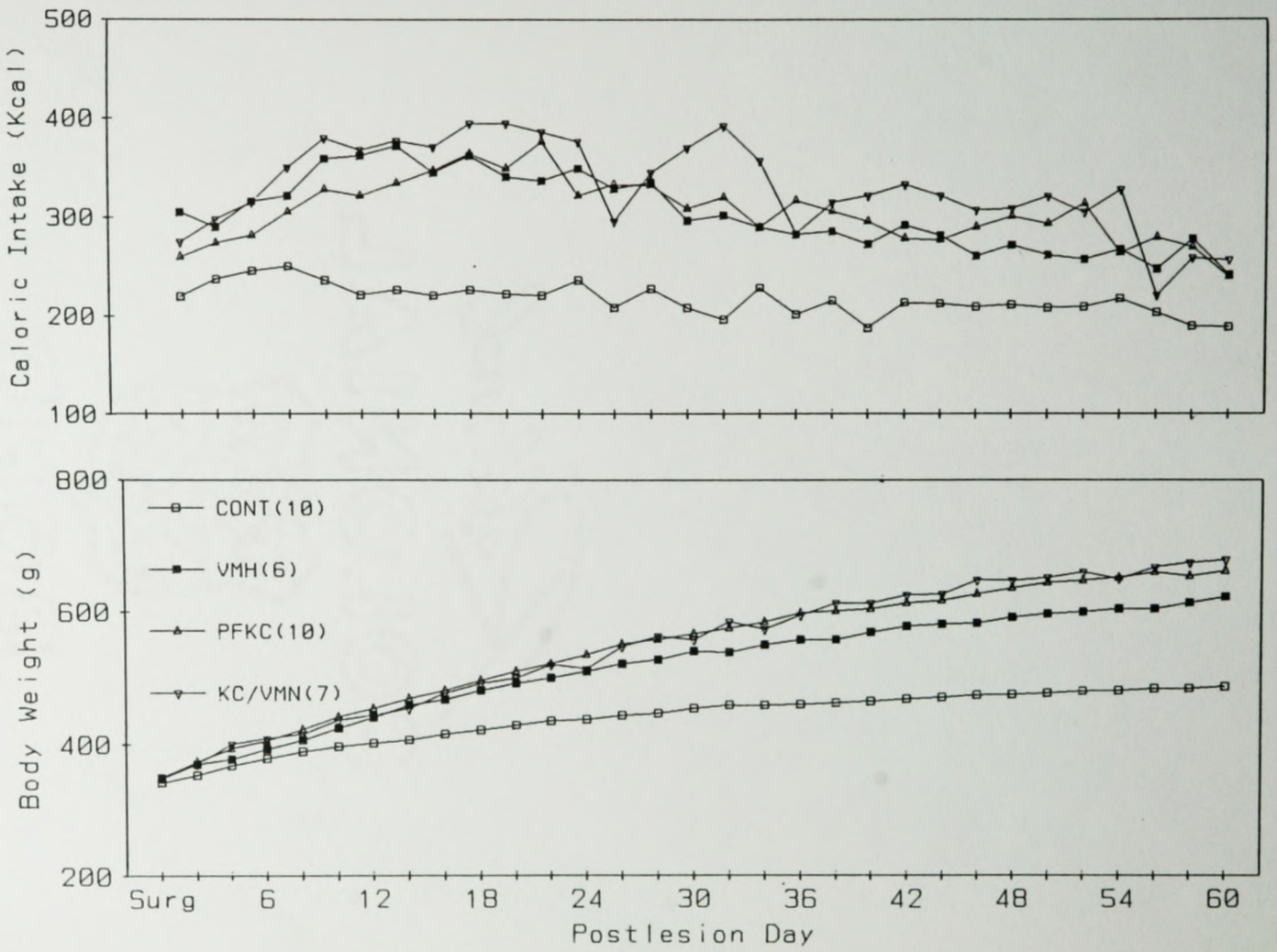
the nucleus. However, the rat was included because knife cuts could be seen to extend at least to the top of the nucleus, were lateral to VMN, and transected the entire region along the anterior-posterior length of VMN. VMN lesions clearly produced considered damage to VMN and remained within the boundaries of the nucleus.

Caloric Intake and Body Weight

Two-day body weight and caloric intake data are shown in Figure 11. Since it was initially considered that the combined sham lesion procedure used in the SHAM-L/SHAM-KC group might affect dependent measures, the two control conditions were included as separate groups in data analyses. However, since the results of data analysis indicated that control groups were not different on mean 24 hr caloric intakes (CONT Mean = 106.5 Kcal/24 hr; SHAM-L/SHAM-KC Mean = 110.8 Kcal/24 hr) or weight gain over the experiment (CONT Mean = 144.0 g; SHAM-L/SHAM-KC Mean = 148.6 g), the data from all control subjects are shown as single 2 day means in Figure 11.

One way ANOVA on subjects' mean 60 day caloric intake scores found a significant group effect $F(4,28) = 15.07$, $p < .001$. Multiple comparisons indicated that VMH, PFKC and PFKC/VMN rats were hyperphagic relative to CONT ($p < .01$ for all comparisons) and SHAM groups ($p < .01$ for all comparisons). The present experiment did not find the significantly greater caloric intake in PFKC rats relative to VMH rats observed in Experiment 1a. However, there was a trend towards higher mean caloric intakes in the PFKC group relative to VMH rats (PFKC mean = 160 Kcal/24 hr, VMH mean = 152 Kcal/24 hr). Importantly, visual examination of Figure 11 and statistical tests indicate that VMN lesions did not significantly

Figure 11. Two-day group mean caloric intakes (Kcal) and body weights over the 60 days of Experiment 1b. CONT indicates the pooled means for animals with either no surgery or combined sham lesions and sham knife cuts. KC/VMN are animals with combined PFKC and VMN lesions.


Caloric Intake (Kcal)
500
400
300
200
100
Body Weight (g)
800
600
400
200
CONT(10)
VMH(6)
PFKC(10)
KC/VMN(7)
Surg
6
12
18
24
30
36
42
48
54
60
Postlesion Day

affect caloric intake or weight gain produced by PFKC lesions (i.e. PFKC/VMN versus PFKC).

The results of analyses on group weight gains over the experiment paralleled the findings for caloric intakes. The groups were not different at surgery, $F(4,28) = 0.77$, N.S. However, the groups differed significantly on day 60 post lesion, $F(4,28) = 9.87$, $p < .001$. Multiple comparisons found VMH, PFKC, and PFKC/VMN groups to be different from each control group ($p < .01$ for all comparisons). There were no other significant group differences. Therefore, PFKC lesions in this experiment did not produce significantly greater weight gains than VMH lesions, although the difference in mean final body weights in the two groups on day 60, shown in Figure 11, suggests a trend towards greater weight gain in PFKC rats. Examination of group mean body weight changes for PFKC/VMN rats shown in Figure 11, and statistical analyses, also indicate that VMN lesions do not reduce weight gain produced by PFKC lesions.

## DISCUSSION

Ventromedial hypothalamic nucleus lesions had no detectable effect on the caloric intake or body weight of rats bearing PFKC lesions. Therefore, damage to VMN neither reduces nor enhances the effect of PFKC lesions on caloric intake or body weight. Consequently, the lower caloric intake in VMH rats relative to the PFKC group in Experiment 1a, is not due to an impairment in the expression of hyperphagia caused by damage to VMN.

Experiment 1a found that PFKC lesions produced greater overeating and weight gain than VMH lesions. Although PFKC rats were slightly more hyperphagic than VMH rats in the present experiment, this difference did

not reach statistical significance. Therefore, PFKC lesions do not reliably produce a greater hyperphagia than VMH lesions.

A simple explanation for the failure of PFKC lesions to produce greater effects on caloric intake and weight gain is not apparent. One difference between the two experiments is that three test diets were used in Experiment 1a, but rats were maintained only on the Mash diet in the present Experiment. Therefore, the relative hyperphagia by PFKC lesions compared to VMH lesions in the previous experiment may be explained by differences in test diets. For example, the relatively greater hyperphagia of PFKC rats relative to the VMH group was most dramatic on High Fat, somewhat lower on Mash, and the groups were not significantly different on the Powder diet. It is possible that PFKC rats would have been more hyperphagic than VMH animals in the present experiment if animals were maintained on the High Fat diet.

A second possibility is that hyperphagia is delayed in knife cut rats relative to VMH rats. A delayed hyperphagia may explain the relatively greater overeating by PFKC rats on High Fat relative to the VMH group and relative to their own intakes on Powder. Furthermore, it is possible that continued measurement of caloric intake in Experiment 1b would have resulted in an overall greater hyperphagia in PFKC rats relative to the VMH group. However, any clear interpretation of the time course of the development of hyperphagia in Experiment 1a is contaminated by the change in test diets over time.

In conclusion, the present experiment indicates that damage to the VMN neither increases, nor decreases, the effects of PFKC lesions on caloric intake and body weight gain. Furthermore, since PFKC lesions did not produce a significantly greater caloric intake or weight gain than VMH

lesions in this experiment, PFKC lesions do not consistently produce greater overeating or body weight changes than VMH lesions. It remains possible however, than PFKC lesions and VMH lesions have subtle differences in their effects on eating behaviour such as differences in the latency of hyperphagia, or in the response to variations of dietary composition.

# CHAPTER 4. EFFECTS OF VMH, PFKC, AND VMN LESIONS ON GASTRIC ACID SECRETION AND PERCENTAGE BODY FAT UNDER RESTRICTED FEEDING CONDITIONS.

## INTRODUCTION

The purpose of this experiment was to determine the relative effectiveness of VMH, PFKC, and VMN lesions for producing obesity, and changes in parasympathetic tone, when overeating is prevented. VMH lesions produce obesity and elevated parasympathetic tone even in the absence of hyperphagia and inordinate weight gain. Therefore, VMH lesions are considered to have "primary" effects on metabolic variables. To determine the effectiveness of PFKC and VMN lesions in producing primary changes in body fat and parasympathetic tone, the effects of excessive food intake and body weight gains on metabolic parameters were minimized by restricting the food intake of lesion animals to maintain all groups at similar body weights throughout this experiment.

Since body compartment changes have not been determined following PFKC lesions, it is not known whether PFKC lesions produce a "metabolic" obesity. It is also not clear whether PFKC lesions produce a primary disturbance in parasympathetic tone. Previous reports indicating an absence of hyperinsulinemia in PFKC rats that are prevented from overeating suggest that vagal tone is unchanged by PFKC lesions. However, measurement of other digestive secretions have not been undertaken to evaluate the

generality of this finding. If obesity and elevated digestive secretions following PFKC lesions are a result of overeating, and not a primary disturbance in metabolism, then PFKC lesions should not produce metabolic changes when hyperphagia is prevented.

The findings of Experiment 1a indicate that VMN lesions increase percentage body fat in the absence of any spontaneous hyperphagia. The following experiment attempts to replicate this finding under restricted feeding where even subtle or transient changes in weight gain (such as those that occurred on High Fat in Experiment 1a) are eliminated in VMN rats. The effect of VMN lesions on digestive secretions have not been determined in mature animals. If damage to VMN produces the metabolic disturbances characteristic of VMH lesions, then VMN lesions should result in elevated digestive secretions. This result would also be expected if vagal tone mediates "metabolic" obesity. Alternatively, metabolic obesity and elevated parasympathetic tone may be independent disturbances and therefore, VMN lesions would produce obesity but no change in digestive secretions.

Measurement of basal gastric acid secretion was used as an index of parasympathetic (vagal) tone. Although insulin secretion has been implicated more strongly in the etiology of VMH obesity, measurement of acid secretion provides a number of advantages over insulin assay. Specifically, basal (or background) gastric acid secretion was chosen as a marker of changes in vagal activity for the following reasons.
(1) Gastric acid secretion has been used frequently as an index of parasympathetic disturbance in VMH lesion rats (Inoue & Bray, 1977; Powley & Opsahl, 1974; Ridley & Brooks, 1965; Weingarten & Powley, 1980;

Weingarten & Parkinson, 1988), and in rats with other hypothalamic ablations (Sawchenko & Gold, 1981; Weingarten et al., 1985).

(2) Gastric acid secretion is highly dependent on the vagus nerve. Vagally mediated reflexive acid secretion is routinely used as a test for completeness of vagotomy in humans (Hollander, 1946) and rats (Snowdon & Epstein, 1970; Burge & Vane, 1958). Vagotomy dramatically lowers both basal and stimulated acid secretion in control rats and eliminates hypersecretions in rats with hypothalamic lesions (Sawchenko & Gold, 1981; Powley & Opsahl, 1974). Furthermore, atropine methyl nitrate, a cholinergic antagonist that interrupts vagal control over digestive responses, can reduce basal acid secretion to near "0" levels (Weingarten & Parkinson, unpublished observations) indicating the dependence of basal acid secretion on the vagus nerve.

(3) Gastric acid secretion is particularly easy to measure in the unanesthetized state and avoids potential methodological artifacts that may result from the procedures used to measure digestive secretions. Previous studies measuring insulin levels in PFKC rats have used procedures that require handling immediately prior to blood sampling, and only a single sample is obtained. Furthermore, the interventions used to obtain blood (i.e. decapitation, or tail cut) would be expected to elicit a strong sympathetic discharge that could inhibit parasympathetic responses. The procedure used by the present experiment permits repeated acid sampling, and samples are taken over a two hour period so that any transient influence of handling on acid secretion is minimized.

(4) Changes in gastric acid secretion parallel changes in insulin and other digestive secretions such as pepsin (Ridley & Brooks, 1965) and gastrin (Chikamori et al., 1983) following hypothalamic lesions. Weingarten and

Powley (1980) noted a number of comparisons between the acid secretion profiles of VMH lesion rats in their study and the findings of others on insulin secretion in VMH rats including: (a) similar time courses for the development of gastric acid hypersecretion and hyperinsulinemia (Hales & Kennedy, 1964), (b) elevated acid secretion, like hyperinsulinemia develops only in animals that become fat (Frohman, Goldman, Schnatz, & Burek, 1969), (c) elevated acid secretion and hyperinsulinemia do not require hyperphagia (Frohman & Bernardis, 1968), and (d) VMH lesions elevated both acid and insulin secretions by about 250 percent (Bernardis & Frohman, 1970).
(5) Finally, gastric acid secretion unlike basal insulin secretion, is not lowered by restricted feeding regimens. The moderate levels of fasting used in restricted feeding studies can reduce insulin levels in VMH rats to normal levels (Bernardis & Goldman, 1972) but do not reduce basal acid secretion (Karakash, Hustvedt, Lovo, LeMarchand & Jeanrenaud, 1977). This may account for previous failures to demonstrate hyperinsulinemia in food restricted PFKC rats. Furthermore, since previous studies have not included VMH lesion animals as a comparison group, it is not clear that the restricted feeding procedures that eliminated hyperinsulinemia in PFKC animals would not have also eliminated hypersecretion in VMH animals. The measurement of gastric acid secretion levels, in both VMH and PFKC lesion rats under restricted feeding, should resolve both of these problems.

## METHOD

Seventy eight rats were implanted with gastric cannulae under sodium pentobarbitol anesthesia. Two animals did not recover from gastric surgery as indicated by weight loss rather than weight gain following surgery.

Three rats developed leakage around the gastric cannula during secretion tests and were removed from the experiment. One rat having sham lesions remained healthy for 11 days following the last secretion trial then started to lost weight rapidly. This animal was included in analyses on gastric secretion but not on carcass composition. Due to the restrictive histological criteria, only 22 animals were identified as having successful lesions leaving a total 34 subjects, with; SHAM-L = 6, SHAM-KC = 6, VMH = 6, PFKC = 6, and VMN = 10.

Following two weeks recovery, measurement of basal gastric secretion was begun. Gastric secretions were collected for 2 hours on 4 prelesion and 6 postlesion trials, spaced 2 days apart. Postlesion trials began on the second day after brain surgery. Since surgery and monitoring of acid secretion was limited to about 12 animals per day, animals received surgery and gastric secretion tests in smaller groups balanced for experimental conditions.

We have found that gastric secretion is highly variable in unanesthetized rats both between subjects and across trials. Variability in secretion levels is particularly problematic in this experiment because of the large number of experimental conditions being compared. To address this problem, prelesion baseline trials were conducted prior to lesion surgery. This permitted animals to adapt to the procedure, and provided an index of secretion that was used to balance groups on acid secretion levels prior to lesions. Furthermore, prelesion baseline trials permitted evaluation of the relative change in secretions produced by different lesions, in addition to between group comparisons made postlesion.

Rats underwent one of four lesion surgeries; SHAM, VMH, PFKC, or VMN defined in General Methods. Postlesion, all VMH, PFKC, and VMN rats were

maintained at control body weights in the following manner. Subjects were weighed and fed daily. Lesion rats were given a food ration sufficient to maintain them at the body weight of a control partner, matched for weight at surgery.

Following gastric secretion trials, rats were maintained on restricted feeding until 40 days postlesion. At this time, all rats were sacrificed and carcasses were analysed for percentage fat in the manner described in General Methods.

## RESULTS

### Histological Findings

The criteria used to define successful and unsuccessful hypothalamic ablations were described in General Methods. The accepted ranges for lesions included in Experiment 1a were adopted and maintained, in addition to the criteria described in General Methods for including lesion animals in data analyses. Therefore, the following description of histological findings only identifies the variability among animals in the specific locus and extent of lesions of animals included in data analysis.

### Ventromedial Hypothalamic Lesions

As in Experiment 1, VMH lesions extended laterally to the fornix but none were observed to reach the lateral hypothalamic area. The fornix remained visible in all but one animal. In only one rat, lesions were comparatively small and were restricted rostrally just anterior to VMN and

caudally just posterior to VMN, but extended laterally beyond the nucleus. One animal had lesions that were dorsal and, therefore, the ventral VMH area may have been spared.

Parafornical Hypothalamic Knife Cut Lesions

As in Experiment 1, PFKC lesions were successful in producing cuts that transected the area between VMN and the fornix, from the top of the third venticle to the base of the brain. All cuts passed laterally to the entire extent of VMN. In all but three animals, knife cuts started rostrally, at the anterior aspect of the PVN. In 2 animals, cuts extended anteriorly as far as the suprachiasmatic nucleus. In one rat, cuts extended, rostrally, only to the posterior aspect of PVN.

As in Experiment 1, PFKC lesions were less symmetrical than VMH lesions. However, in no case could bilateral damage to VMN, fornix, or LHA be detected. In one animal, the third ventricle was distended unilaterally possibly producing some unilateral damage to VMN or DMH.

Ventromedial Hypothalamic Nucleus Lesions

VMN lesions were highly symmetrical bilaterally and well centered in the nucleus in their medial-lateral orientation. In four rats, lesions were oriented rostrally so that they started at the border between VMN and the anterior hypothalamus. Lesions extended as far caudally as the posterior border of VMN in one animal. The fornix and base of the brain remained observable in all brains.

Body Weight Changes

Figure 12 displays group mean body weights at surgery and each postlesion day until sacrifice. The groups were not significantly different in body weight at surgery [$F(3,31) = 0.09$, N.S.] or on the final day of the experiment [$F(3,30) = 0.09$, N.S.]. The restricted feeding procedure was successful in maintaining the body weights of all lesion groups to be similar to sham lesion rats over the acid sampling period. An analysis conducted on body weights during the 12 days over which acid secretion was sampled, found no group differences [$F(3,30) = 0.55$, N.S.] nor any significant Group x Trials interaction [$F(33,330) = 0.98$]. To compare groups on average body weights over the entire experiment, a mean post-lesion body weight was calculated for each subject over the 38 postlesion days. There were no significant group differences in subject's mean body weights over the experiment [$F(3,30) = 1.39$, N.S.].

Gastric Secretions

To determine the effects of different hypothalamic lesions on gastric secretion parameters, 2 hour basal gastric secretion trials were conducted pre-, and post-lesion. Prelesion baseline trials were conducted until acid secretions proved stable, resulting in 4 prelesion trials. Postlesion secretions were collected for 6 trials.

a. Stability of Gastric Secretion

Figure 13 displays group mean secretion scores for all test trials. The first set of analyses performed on gastric secretion parameters determined the degree of stability of secretions, and stability of group differences, over prelesion and postlesion acid test trials. There was no

Figure 12. Comparison of daily group mean body weights over the 40 days in Experiment 2. For comparison, VMH-A shows the two day group mean body weight for VMH lesion rats in Experiment 1a. Weight fluctuations occuring between the day prior to surgery (Surg) and day 12 postlesion were caused by the 17 hour deprivation imposed before gastric secretion trials.

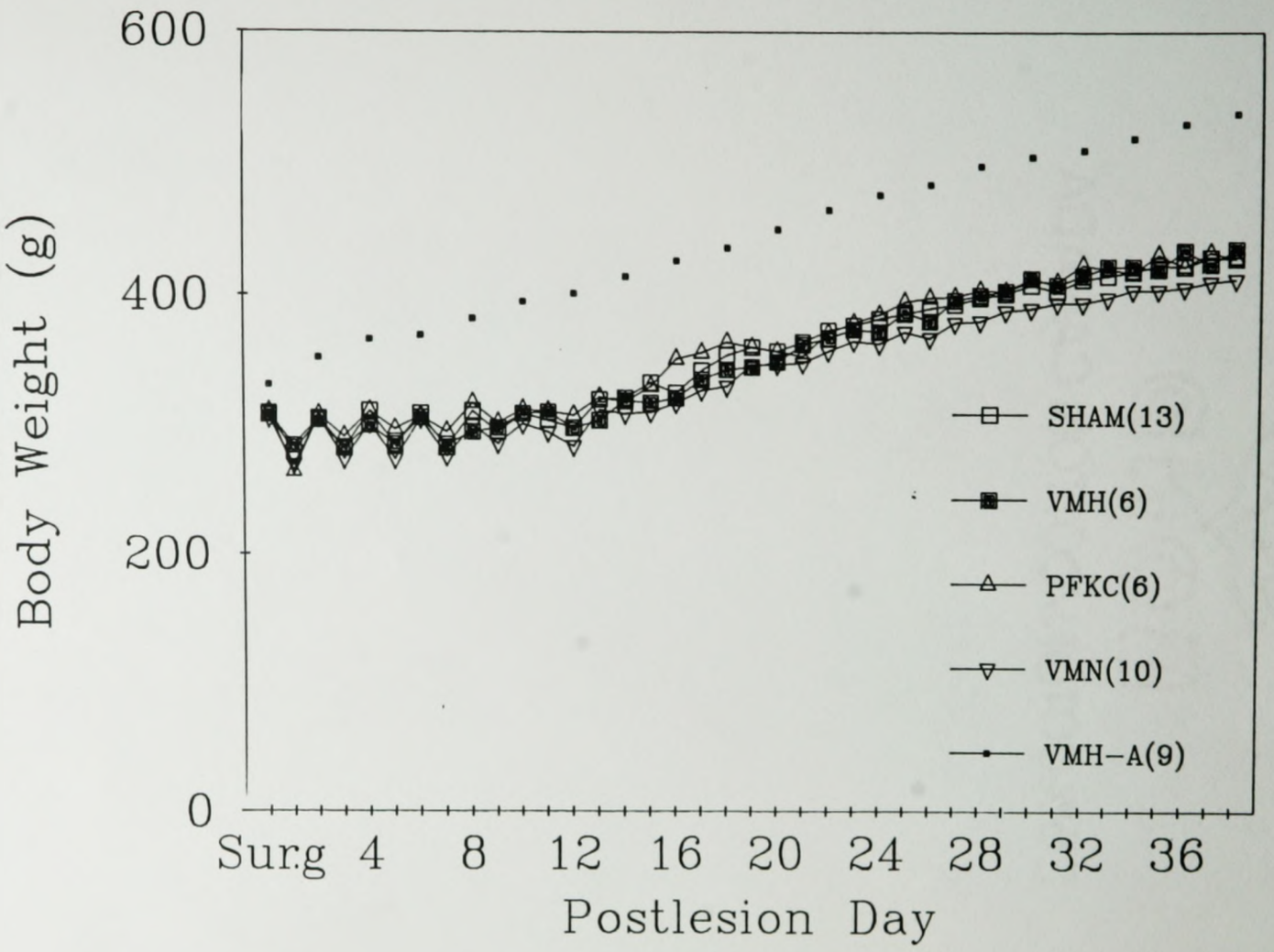
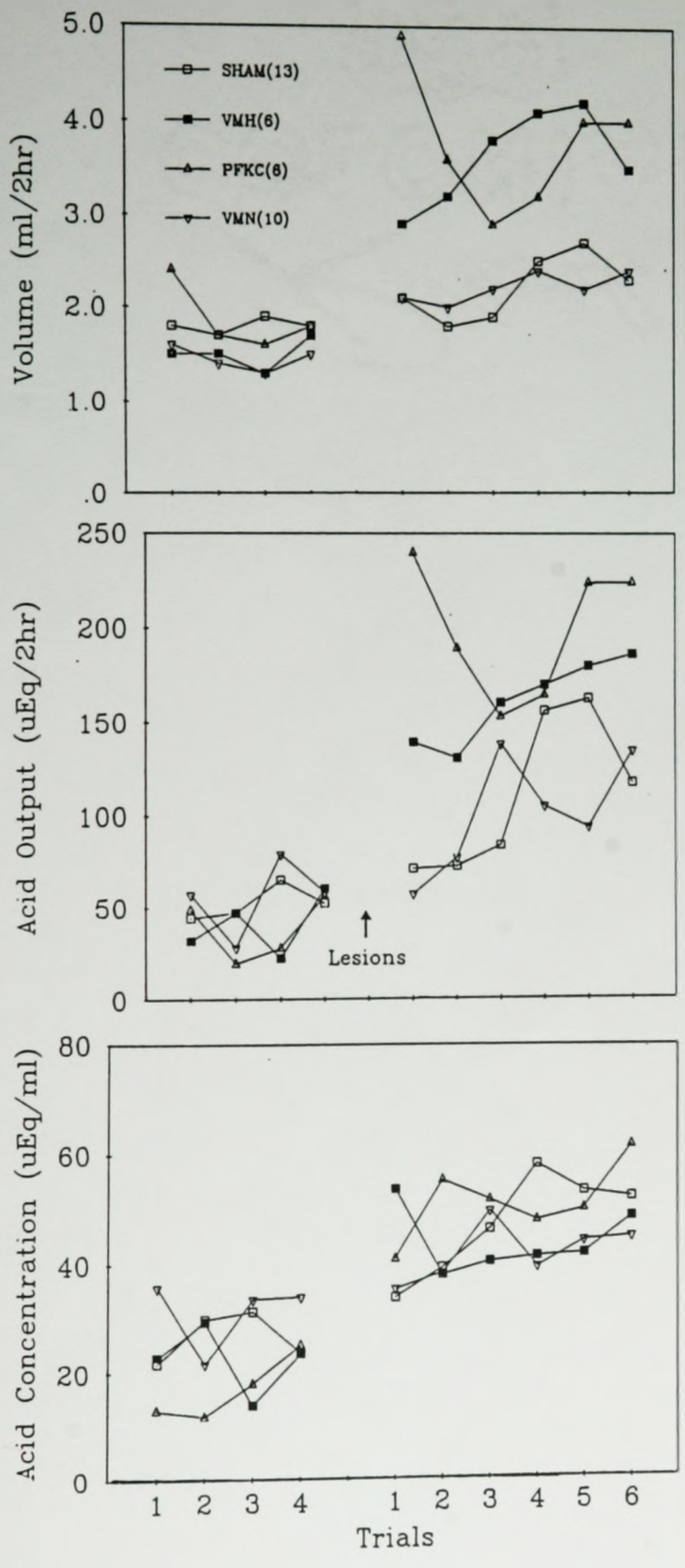

600
400
200
0
Body Weight (g)
Surg 4 8 12 16 20 24 28 32 36
Postlesion Day
SHAM(13)
VMH(6)
PFKC(6)
VMN(10)
VMH-A(9)

Figure 13. Group mean gastric secretion parameters on pre-, post-lesion acid test trials.


SHAM(13)
VMH(6)
PFKC(6)
VMN(10)
Volume (ml/2hr)
5.0
4.0
3.0
2.0
1.0
.0
Acid Output (uEq/2hr)
250
200
150
100
50
0
Lesions
Acid Concentration (uEq/ml)
80
60
40
20
0
1 2 3 4
1 2 3 4 5 6
Trials

significant effect for Time nor any significant Group x Time interactions, before lesions. The only evidence of a time effect postlesion was a main effect for trials on acid output [$F(5,155) = 2.30$, $p < .05$] and a Group x Trials interaction for volume [$F(15,155) = 1.94$, $p < .05$)]. A simple effects F test found the source of this interaction to be the increase in volume over trials in the VMH rats relative to SHAM controls [$F(5,155) = 6.62$, $p < .001$]. The increase in acid output over trials was consistent between groups, as indicated by the absence of a Group x Time interaction on that measure.

b. Effects of Lesions on Gastric Secretion.

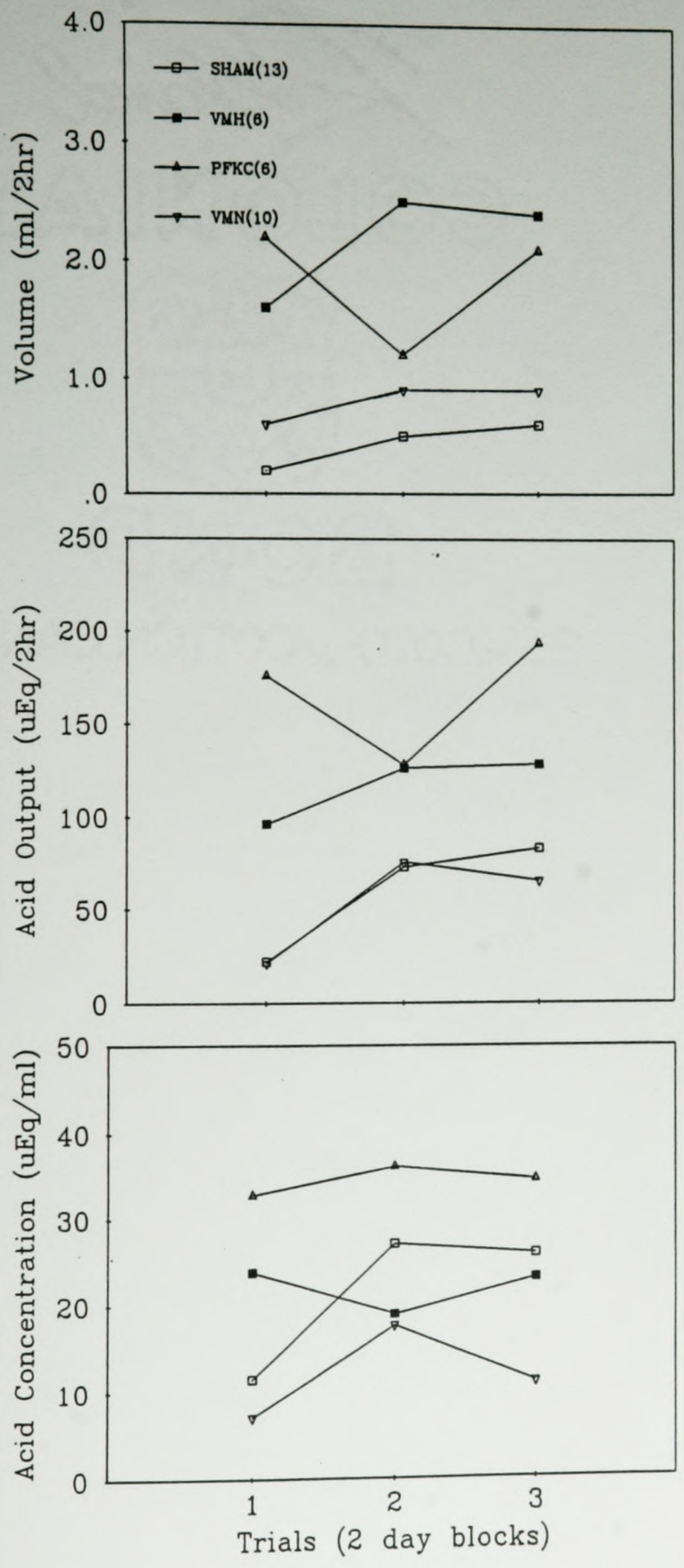
There were no significant group differences before lesions on secretion volume [$F(3,31) = 0.52$, N.S.], acid output [$F(3,31) = 0.39$, N.S.], or acid concentration [$F(3,31) = 0.73$, N.S.].

To compare lesion effects on acid secretion, for each subject, the pre-, to post-lesion change in volume, output and concentration scores on the 6 postlesion trials were expressed as a change from each subject's baseline secretion (the mean of the 4 prelesion secretion trials). These change scores are shown in Figure 14.

Analysis of change scores revealed group differences on volume [$F(3,155) = 6.96$, $p < .001$], acid output [$F(3,155) = 6.32$, $p < .01$], but not acid concentration. However, there was a trend towards group differences on acid concentration [$F(3,155) = 2.36$, $p = .09$]. There were no significant effects of trials, or any Group x Trials interactions.

Multiple comparisons indicated that, relative to SHAM controls, VMH lesions elevated volume ($p < .01$) and acid output ($p < .05$). PFKC rats were also elevated on volume and acid output relative to the SHAM group

Figure 14. Group mean prelesion to postlesion changes in gastric secretion parameters on postlesion acid test trials. Data points are 2 day blocks, where Blocks 1, 2, and 3 are Postlesion days 1-2, 3-4, and 5-6, respectively.


SHAM(13)
VMH(6)
PFKC(6)
VMN(10)
Volume (ml/2hr)
4.0
3.0
2.0
1.0
.0
Acid Output (uEq/2hr)
250
200
150
100
50
0
Acid Concentration (uEq/ml)
50
40
30
20
10
0
1
2
3
Trials (2 day blocks)

($p < .01$ for both comparisons). The VMN and SHAM groups did not differ on volume or acid output.

Multiple comparisons among lesion groups found VMH lesions to elevate secretion volume ($p < .05$) relative to VMN lesions. PFKC lesions elevated both volume ($p < .05$) and acid output ($p < .01$) relative to VMN lesions. VMH and PFKC groups were not different on any of the secretion measures.

Carcass Composition

The results of the carcass analysis are located in Table 4. Given the restricted feeding regimen, it is not surprising that there were no significant group differences in carcass weights [$F(3,30) = 2.63$, N.S.]. However, the groups differed in percentage carcass fat [$F(3,30) = 32.04$, $p < .001$]. Post hoc comparisons found VMH and VMN lesions to produce increased percentage body fat relative to both SHAM and PFKC lesions ($p < .01$ for all comparisons). VMH rats also had a greater percentage fat than VMN rats ($p < .01$). PFKC lesions did not produce a significant elevation in percentage fat relative to SHAM controls.

The level of obesity in SHAM rats was considerably lower (13.0%) compared to Experiment 1a (22.9%). This could have been due to a number of factors including: the fasting required for acid sampling, the shorter duration of the experiment, or the dietary manipulations used in Experiment 1a. The final mean carcass weight for SHAM rats in Experiment 1a (440.0) was considerably higher than that observed in this study (339.6), possibly because of greater fat deposition in the former experiment. The obesity produced by VMH lesions was also reduced in this experiment (31.7%) relative to ad libitum feeding (52.6%). However, degree of elevation in carcass fat over SHAM rats is consistent between ad libitum conditions in

Experiment 1a (230%) and the restricted feeding conditions employed in this study (244%).

Table 4. Group Mean Carcass Composition Measures For Rats Maintained on Restricted Feeding.

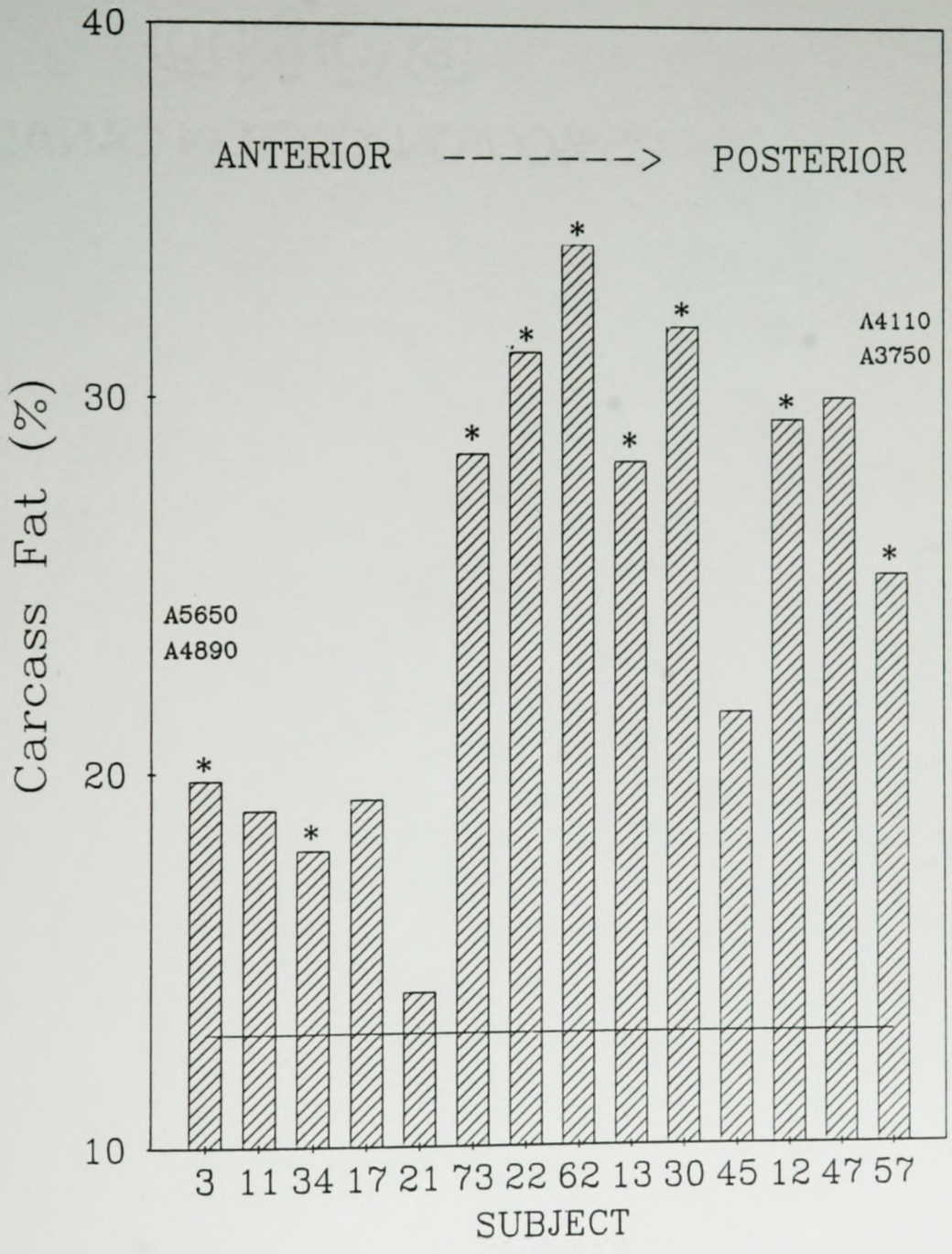
| GROUP | CARCASS WEIGHT (g) | PERCENT WATER | PERCENT FAT |
|---|---|---|---|
| SHAM (12) | 339.6 (7.9) | 65.2 (0.6) | 13.0 (0.8) |
| VMH (6) | 360.7 (4.4) | 50.5 (1.3) | 31.7 (1.7) |
| PFKC (6) | 359.5 (9.1) | 62.7 (1.0) | 16.2 (1.3) |
| VMN (10) | 332.4 (8.6) | 55.2 (1.5) | 25.7 (1.9) |

Numbers in parentheses are 1 standard error of the mean.

Relationship Between Lesion Locus and Obesity

To examine the role of VMN in the control of adiposity more closely, animals were ranked according to the observable anterior extent of the lesion (i.e. the same procedure used to examine the relationship between caloric intake and lesion locus in Experiment 1a). Figure 15 depicts the results of this analysis. All rats had some damage to the nucleus. However, only in rats 34 - 57 did the lesion start at the most anterior aspect of the nucleus, as determined by reference to control brains, and using Konig and Klippel's (1963) atlas of the rat brain. With reference to

Figure 15. Individual percent carcass fat for VMN lesion rats (accepted and not accepted) ranked according to the anterior border of the lesion. Horizontal line is the mean for SHAM rats. Asterisks indicate animals accepted as successful lesions.


40
30
20
10
Carcass Fat (%)
ANTERIOR -------> POSTERIOR
A5650
A4890
A4110
A3750
3 11 34 17 21 73 22 62 13 30 45 12 47 57
SUBJECT

the coordinates used in Konig and Klippel (1963), in subject 3 the lesion started at A5650 and ended at A4890. In subject 11 the lesion began at A5340 and ended at A4620. Therefore, the lesion only damaged the most anterior aspect of VMN in these animals. In subjects 34 and 17, the lesion started at or near the most anterior aspect of VMN (A5150) and extended to about A4380. In subject 21 the lesion began at A4890 and ended at A4110. However, the lesion was relatively high and could have spared the ventral aspect of VMN. This may explain the relatively lower percentage carcass fat in this animal. Remaining animals (73-57) had lesions that started well within VMN. Subjects 73 and 22 had lesions that started at A4620 and ended at A3990. The lesion in subject 57 began at A4110 and ended at the rostral aspect of VMN (A3750). Therefore, no lesions extended into the posterior hypothalamus.

## DISCUSSION

This experiment provided the following novel findings that support a dissociative analysis of the VMH obesity syndrome. The essential findings for a dissociative analysis are summarized in Table 5.

(1) Parafornical hypothalamic knife cut lesions do not produce obesity when food intake is restricted and excessive weight gain is prevented (i.e. metabolic obesity).

(2) PFKC lesions result in a primary elevation in vagal tone, as indexed by gastric acid secretion.

(3) VMN lesions do not elevate vagal tone. In addition, this experiment replicates the metabolic obesity observed in VMN lesions rats in Experiment 1a.

Table 5. Results of Experiment 2.

| GROUP | GASTRIC SECRETION | %FAT |
|---|---|---|
| VMH | + | + |
| PFKC | + | - |
| VMN | - | + |

Symbols represent significant differences from control rats. Plus indicates increase. Dash indicates no change.

The primary elevation of body fat produced by VMH lesions is well documented. However, this experiment makes the first observation that PFKC lesions do not produce this metabolic abnormality. Until this study, PFKC obesity has been defined and measured as an increased body weight. Measurement of actual changes in body composition not only provides a more definitive measure of obesity, but leads to an important distinction between VMH and PFKC obesity syndromes. Obesity produced by PFKC lesions depends on overeating, obesity produced by VMH lesions does not.

Since PFKC lesions produce no metabolic obesity, but VMH lesions do, VMH lesions must disturb additional mechanism(s) that influence fat deposition. Furthermore, since PFKC lesions were not as effective as VMH lesions for producing increases in percentage body fat on ad libitum

feeding, disturbance of metabolic mechanisms by VMH lesions contributes to the level of obesity that results from overeating. However, the relative increment produced by this metabolic effect over that produced by hyperphagia is small.

The production of obesity by VMN lesions in the present experiment strengthens the conclusion made in Experiment 1 that VMN lesions produce obesity in the absence of hyperphagia. However, the present experiment is a more controlled demonstration of this primary disturbance because it produces the same effect under conditions in which any overeating or weight gain was eliminated. In fact, the mean body weight of VMN rats in this experiment was slightly less than SHAM controls by the end of the study. Therefore, this effect cannot be attributed to a subtle hyperphagia, or to the elevated rate of weight gain observed on High Fat in Experiment 1a. Moreover, the absence of metabolic obesity in PFKC rats indicates that increased percentage carcass fat in VMN rats cannot be attributed to inadequacies in the restricted feeding procedure as this procedure clearly eliminated obesity dependent on overeating.

Although VMN lesions produced a metabolic obesity, this was not accompanied by an increased vagal tone, as indexed by gastric acid secretion. Instead, PFKC and VMH lesions produced elevated acid secretion. Therefore, elevated parasympathetic tone is neither <u>sufficient</u> nor <u>necessary</u> for metabolic obesity as suggested by metabolic theories (Bray & York, 1979; Powley, 1977). Since metabolic obesity does not result from elevated vagal tone, the mechanism for the primary elevation of body fat by VMN and VMH lesions remains unknown. A second important implication of this finding is that VMN lesions cannot account for all metabolic effects of VMH lesions. A simple dichotomy between behavioural and metabolic

variables would have rendered a dissociative model of the VMH syndrome more parsimonious. However, these data continue to support a dissociative interpretation because they indicate that elevated vagal tone and metabolic obesity are dissociable disturbances resulting from lesions in the area of the VMH. Therefore, separate mechanisms underly these different metabolic processes.

VMN lesions clearly disturb some mechanism(s) involved in the regulation of body fat that is not affected by PFKC lesions. Although the results of this experiment support a role for the VMN in the regulation of body fat, these findings do not prove that it is damage to the VMN per se that produces metabolic obesity. First, it remains possible that the difference between PFKC and VMN lesions on body fat under restricted feeding conditions is due to the type of lesion rather than its locus. For example, electrolytic lesions, unlike knife cuts, leave iron deposits that are reported to have irritative properties (King & Frohman, 1985) which might affect the regulation of body fat. A second difference between VMN and PFKC lesions is that VMN lesions destroy cells whereas knife cuts sever fibres of passage. It is possible that body fat is regulated by cells in the VMH that are not necessarily limited to VMN. The fact that larger VMH lesions produced a greater obesity than VMN lesions is consistent with this hypothesis. However, analysis of the relationship between the anterior-posterior locus of lesions and percentage body fat indicated that obesity was a function of lesion location. Anterior lesions were less effective than posteriorly positioned damage. Although it is tempting to argue that anterior VMN lesions were less effective for producing obesity because they produced less VMN damage, such a conclusion cannot be clearly drawn. Rats 34, 17, and 21 had lesions that began at about the anterior aspect of the

nucleus yet displayed less adiposity than rats with more posterior lesions. Since the nucleus is relatively small at this point, it is likely that these lesions were relatively less effective for producing VMN damage. It remains possible, however, that the relevant site for the effect is more posterior in VMN.

Finally, it is noteworthy that the anterior-posterior locus of VMN lesions was relevant to their effect on body fat but not to feeding, as indicated in Experiment 1a. This difference provides further evidence for a dissociation of metabolic and behavioural mechanisms.

In summary, the present experiment provides novel findings that support a dissociative perspective of the VMH obesity syndrome. PFKC lesions, that were found in Experiments 1a and 1b to be as effective as VMH lesions for producing hyperphagia and weight gain, did not produce metabolic obesity. Since VMH lesions clearly produced a primary elevation in percent body fat, it is concluded that separate mechanisms underly the hyperphagia and metabolic obesity characteristic of VMH lesions. It is also concluded that metabolic obesity and elevated parasympathetic tone are dissociable disturbances resulting from medial hypothalamic damage.

# CHAPTER 5. GENERAL DISCUSSION

The purpose of this thesis was to determine the potential for a dissociative perspective of the ventromedial hypothalamic obesity syndrome. By providing novel observations on behavioural and metabolic changes following PFKC and VMN lesions, and by comparing the relative effectiveness of PFKC and VMN lesions for producing these changes, these experiments have demonstrated that major behavioural and metabolic components of the VMH syndrome can be anatomically dissociated. These observations also corroborate previous findings that implicate the VMN in the control of adipose tissue stores, and a parafornical hypothalamic fibre system in the control of food intake. In general, these data support a dissociative perspective of the VMH obesity syndrome and therefore have important theoretical implications for the study of the anatomy and etiology of hypothalamic obesity.

## Basic Observations

This thesis contributed the following novel observations.

(1) PFKC rats become obese when permitted to overeat.

(2) PFKC rats do not become obese when excessive food intake is prevented.

These findings, collectively, indicate that PFKC lesions produce obesity by

overeating but not by a metabolic disturbance.

(3) VMN lesions produce obesity in the absence of a spontaneous hyperphagia, and when body weight gain is held at the rate of control rats. Thus the obesity resulting from VMN lesions is a "metabolic obesity".

(4) VMN lesions do not elevate parasympathetic tone, as indicated by the absence of any elevation in basal gastric acid secretion.

(5) PFKC lesions produce a primary elevation of parasympathetic tone, as indicated by elevations in basal acid secretion even when excessive food intake and weight gain are prevented.

(6) VMH lesions produce a slightly, but significantly, greater obesity than PFKC lesions when animals feed ad libitum.

(7) The combination of PFKC lesions and VMN lesions does not result in a greater, or lesser, hyperphagia and weight gain than PFKC lesions alone.

In addition to these novel observations, this thesis replicates and expands on a number of previous findings. First, VMH lesions produce hyperphagia, metabolic obesity, and elevated digestive secretions. Second, VMN lesions do not produce a spontaneous hyperphagia. It was also demonstrated in Experiment 1a that VMN lesions do not produce hyperphagia on three test diets. Therefore, previous demonstrations finding an absence of changes in caloric intake following VMN lesions are not specific to the test diet used. Finally, VMH and PFKC lesions both produced hyperphagia and weight gain. The finding that PFKC lesions are at least as effective as VMH lesions for producing overeating and body weight increases, replicates a previous observation (Sclafani & Aravich, 1983). However, the present study also demonstrated that PFKC lesions remained as effective as VMH lesions for producing these changes on three test diets.

Dissociation of Hyperphagia and Metabolic Obesity

Experiment 1a and Experiment 2 considered together, indicate that hyperphagia and metabolic obesity are anatomically dissociable effects of lesions made in the ventromedial hypothalamus. When excessive food intake and body weight gains are prevented by restricted feeding, VMH and VMN, but not PFKC, rats become obese. Therefore, only VMH and VMN lesions produce a metabolic obesity. When permitted to eat ad libitum, PFKC and VMH, but not VMN, rats are hyperphagic. Therefore, only VMH and PFKC lesions produce overeating. It is concluded then, that PFKC lesions produce hyperphagia but not metabolic obesity, while VMN lesions produce metabolic obesity but not hyperphagia.

This is the first study that measures the effects of PFKC lesions on percentage body fat and, therefore, the first study that determines directly whether PFKC lesions produce obesity. PFKC lesions did not produce obesity on restricted feeding. However, the results of Experiment 1a indicate that PFKC rats can become obese if permitted to overeat. Therefore, unlike VMH and VMN rats, PFKC rats become obese by consuming more calories but not by a primary metabolic effect.

This thesis replicates the well cited finding of Gold (1973) that VMN lesions do not produce a hyperphagia. A number of other observations made in this study further support the conclusion that damage to VMN does not produce or contribute to hypothalamic hyperphagia. First, hyperphagia did not develop in VMN lesion rats on a variety of test diets (Experiment 1a). Second, PFKC lesions, made outside VMN, produced overeating and weight gain equal to (Experiment 1b) or greater than (Experiment 1a) VMH lesions. Therefore, damage to VMN is not necessary for maximum hypothalamic

hyperphagia. Finally, VMN damage did not affect the caloric intake or weight gain of rats bearing PFKC lesions (Experiment 1b).

The finding that VMN lesions produce obesity is the first demonstration of an elevation in body fat by lesions of the ventromedial nucleus in the mature animal. Prior to the present study, evidence for a hypothalamic obesity independent of hyperphagia has come from two observations. First, some studies have demonstated obesity in VMH rats when hyperphagia is prevented (Han, 1967; Powley & Opsahl, 1974). In such studies it can only be assumed that the restricted feeding procedure has been successful in completely eliminating the effects of overeating on body fat. In contrast, the present thesis demonstrates that obesity can occur in VMH and VMN lesioned rats on a restricted feeding schedule that is concurrently demonstrated to be effective in preventing obesity due to overeating by PFKC lesions.

A second form of evidence has relied on observations made in weanling rats. Relatively small VMN lesions, which do not produce hyperphagia in weanlings, still produce increased percentage carcass fat (Frohman & Bernardis, 1968). Although, this finding does indicate that behavioural and metabolic effects of hypothalamic lesions are functionally dissociable, the finding does not necessarily imply separate anatomical mechanisms in the mature animal. Since larger lesions also produce elevated percent carcass fat, but no hyperphagia in weanlings (Han, 1967; Kennedy, 1957), VMH influence over eating may not be developed in the weanling. It is therefore unclear whether a separate mechanism for hyperphagia develops in the hypothalamus, or metabolic changes by VMH lesions begin to influence feeding only with maturity. Finally, because weanlings consume about twice as many calories per day, relative to their body weight, compared to adult

rats, it is possible that failure to obtain hyperphagia in weanlings is due to a ceiling effect. Only the present findings clearly indicate that separate hypothalamic mechanisms must underly hyperphagia and obesity following VMH lesions in the mature rat.

Previous research findings of an absence of primary metabolic disturbances in PFKC rats has been used to support the arguments that the hyperphagia produced by knife cuts is independent of peripheral metabolic changes. In support of this general perspective is the present finding that PFKC rats do not display a metabolic obesity. However, since PFKC lesions continued to produce elevated gastric acid secretion, knife cuts do affect at least one peripheral digestion-related process.

A major question remains. Is the level of obesity observed in VMH rats feeding ad libitum, a function of both the primary metabolic and behavioural effects of the lesion? A definitive answer to this question cannot be provided. However observations made in Experiment 1a suggest that both hyperphagia and metabolic obesity contribute to the observed level of adiposity in VMH rats. First, VMH rats were more obese than VMN rats. Since both VMH and VMN lesions produce a primary obesity, but only VMH rats overeat, this finding is consistent with a role for hyperphagia in determining the degree of obesity. Second, PFKC lesions, which produce no metabolic obesity, resulted in a greater adiposity than VMN lesions. Therefore overeating alone, led to a greater obesity than the primary metabolic disturbance produced by VMN damage. Finally, VMH rats became more obese than PFKC rats in Experiment 1a. The absolute difference in body fat was small between the two groups, but was statistically significant and may have been reduced by the lower overeating in the VMH

group relative to PFKC rats. Therefore, overeating alone does not explain the degree of obesity produced by VMH lesions.

## Dissociation of Metabolic Obesity and Elevated Parasympathetic Tone

This thesis provides the first demonstration of a dissociation of metabolic obesity and elevated digestive secretions. In Experiment 2, PFKC lesions produced a dramatic elevation in gastric acid secretion but no metabolic obesity. Furthermore, the VMN rats in the same experiment showed no elevation of gastric secretion parameters, but displayed marked elevation in percentage body fat relative to SHAM rats and to the PFKC group. Therefore, neither the absence of metabolic obesity in PFKC rats, nor the absence of elevated digestive secretions in VMN rats can be attributed to methodological problems or sensitivity of measures. Rather, it is concluded that the hypothalamic mechanism(s) modulating basal digestive secretions and the mechanism(s) involved in the primary effect of VMH lesions on body fat, are different.

## Mechanisms of Hyperphagia and Metabolic Obesity

The purpose of this thesis was not to identify the mechanisms of metabolic and behavioural components of the VMH syndrome. However, these experiments provide data relevant to delineating the specific anatomical and physiological substrates of these disturbances.

First, these data support the findings of other studies aimed at specifying the anatomical substrate of hypothalamic hyperphagia (Albert, Storlien, Albert, & Mahl, 1971; Grossman, 1971; Sclafani & Berner, 1977). It has been hypothesized that knife cut lesions produce hyperphagia by interrupting a longitudinal nerve fibre system that courses through the VMH

area, just lateral to the VMN, and terminates rostral to VMN at the paraventricular hypothalamic nucleus (PVN). It has also been suggested that this hypothetical system specifically determines behaviour and not metabolic processes (Sclafani & Kirschgessner, 1986; Weingarten et al., 1985). This model was generated following studies that demonstrated that PFKC lesions that produce hyperphagia do not produce hyperinsulimia on restricted feeding regimens (Bray et al., 1982). Further support has been made by studies finding that lesions of the paraventricular hypothalamus cause hyperphagia but do not elevate gastric acid or insulin secretions, and do not produce obesity when hyperphagia is prevented by restricted feeding (Weingarten, et al., 1985). According to the model, these results are interpreted to indicate that PVH and PFKC lesions interrupt the same system involved in the control of food intake at different loci.

The present experiments offer some support for this scheme. First, PFKC lesions produced a dramatic increase in caloric intake and weight gain but like PVH lesions, did not produce a metabolic obesity. Therefore, in both PVH and PFKC rats, obesity results from overeating and not from metabolic disturbances. Accordingly, neither PFKC or PVH lesions produce as dramatic an obesity (i.e. percent fat) as VMH lesions under ad libitum feeding conditions, presumably because metabolic effects of VMH lesions contribute to the level of obesity. Also consistent with this model, was the finding that knife cuts that were either too posterior or too medial, unilaterally, to disturb this hypothetical system were less effective than those placed directly in the path of this system.

However, this study also found that PFKC lesions had effects that contrast with those of PVH lesions. First, PFKC rats displayed gastric acid hypersecretion on restricted feeding, but PVH rats did not (Chang,

1985). Furthermore, in the latter study, which was conducted in the same laboratory and using a similar protocol as the present experiments, PVH rats were found to be less hyperphagic and gain less weight than VMH rats. In this study, PFKC rats were no less hyperphagic than VMH subjects. The mean daily caloric intake of VMH rats on each of the test diets was highly similar between the two studies (Powder = 151 Kcal vs 158 Kcal, Mash = 150 Kcal vs 152 Kcal, High Fat = 131 Kcal vs 138 Kcal, Chang (1985) study vs present study, respectively). Therefore, while the similarities between the effects of PFKC and PVH lesions on food intake could be interpreted to support the disturbance of a common system, differences in the level of overeating and effects on parasympathetic tone indicate that either separate or additional systems may be disturbed by PFKC lesions. Since increased gastric acid secretion occurs with PFKC, but not PVH lesions, it is tempting to speculate that altered digestive activities explain the larger hyperphagia produced by PFKC and VMH lesions relative to PVN lesions. In any case, the possibility remains that altered digestive responses contribute to hypothalamic hyperphagia.

The data reported in this thesis implicate the VMN in the control of adipose stores. Although it has repeatedly been argued that the VMN is involved in the regulation of body fat (Hetherington & Ranson, 1942b; Kennedy, 1952; Han, 1967; LeMagnen, 1983), this thesis contrasts with previous notions that failed to distinguish between the VMH and the VMN. The present data support the hypothesis that it is the VMN per se that is responsible for metabolic obesity and therefore argues in favour of restricting an analyses of a primary role of VMH in the regulation of adipose storage to the role of VMN.

Analysis of the relationship between exact lesion locus and body fat found that more posterior VMN lesions were most effective for producing obesity. One interpretation of this finding is that more rostral lesions produced less damage to the nucleus. Alternatively, it is possible that the relevant site for the production of obesity is the posterior VMN. By either explanation, the relatively greater effect of posterior VMN lesions on body fat indicates that the locus of the lesion is relevant for a metabolic obesity. This finding contradicts the possibility that the differences between PFKC lesions and VMN lesions on body fat are caused by the type of lesion rather than the locus of damage.

It is noteworthy that the anterior - posterior locus of VMN lesions was relevant to their effects of body fat (Experiment 2) but not on caloric intake (Experiment 1a) even though the anterior - posterior range of lesions in these separate analyses was similar. Since more posterior VMN lesions were more effective for obesity but not for producing hyperphagia, these observations provide further support for the dissociation of obesity and overeating.

Since VMN lesions produce obesity but not hyperphagia, the mechanism of metabolic obesity, and the physiological role of VMN, is unclear. The first theory implicating the VMN, directly, in the regulation of adipose stores was suggested by Kennedy (1952). Kennedy argued that lesions of the VMH altered a "set point" for the regulation of body fat. Termed the "lipostatic hypothesis" the argument was that damage to VMN directly elevated the animal's "prefered", homeostatically defended, level of body fat, and that the effects of the lesion on behaviour could be viewed as a regulatory adjustment to the homeostatic imbalance. This idea was attractive because it could explain the vigorous defense of static phase

obesity in VMH rats (Hoebel & Teitelbaum, 1966). The effects of VMH lesions on autonomic responses were not described at that time. However, it is easy to see how the hypothesis could also explain the functional relationship among syndrome components (e.g. hyperphagia, increased digestive activities, impaired lipolysis). According to the hypothesis, these other changes are, like hyperphagia, activated to serve a common goal; namely, increased fat deposition and consequently, the defense of the body fat set point.

A number of observations contradict the lipostatic hypothesis of the VMH syndrome (Keesey & Powley, 1986). For example, VMH obesity is highly dependent on the palatability of the test diet (i.e. finickiness). Therefore, appetitive mechanisms do not function to defend a fixed level of body fat in VMH rats. The present experiments provide additional evidence against this explanation of VMH obesity. While these experiments do not disprove the hypothesis, the parsimony of this explanation is lost when one considers the fact that different lesions in the VMH produce obesity by different mechanisms. PFKC rats become fat by overeating, while VMN rats become fat by some other, hitherto undefined, mechanism. Therefore, VMN lesions do not produce a general elevation in the body fat set point that leads to regulatory adjustments in behavioural (i.e. eating) and physiological (i.e. digestion and metabolism) processes.

The major alternative explanation of metabolic obesity has been to consider changes in body fat to be consequences of exaggerated parasympathetic tone. This hypothesis is contradicted by the dissociation of metabolic obesity and elevated parasympathetic tone reported here. Specifically, since PFKC rats demonstrate an increased vagal tone but no metabolic obesity, increased vagal tone is <u>insufficient</u> to produce obesity.

Furthermore, since VMN lesions produce no increase in vagal tone, but do produce a metabolic obesity, increased vagal tone is not necessary for obesity. The latter finding also indicates that the role of VMN in metabolic obesity is not explained by a general increase in vagal tone.

This finding contributes to a line of evidence that has found the concept of a primary elevated parasympathetic tone to be an insufficient explanation of the VMH obesity syndrome. In other studies conducted by this author (Appendix A), VMH lesions were found to have no consistent effect on vagally mediated reflexive gastric acid secretion. VMH lesions elevate acid secretion to a single dose of 2 deoxy-D-glucose and to low dose insulin, but responses to high dose insulin were reduced. In a second experiment, Pavlovian conditioned acid secretion was eliminated by VMH lesions. Following gastric secretion testing in the latter experiment, rats were permitted to overeat and gain weight. The VMH lesion group displayed a dramatic weight gain relative to brain-intact controls. Therefore, hyperphagia-producing VMH lesions actually impaired vagally mediated gastric acid secretion under certain conditions. That study, and this thesis, together indicate that: (1) VMH hyperphagia and weight gain are not associated with any consistent elevation in phasic parasympathetic responses; (2) the metabolic obesity produced by VMH lesions is not caused by an elevation in tonic parasympathetic responses.

A comment should be made regarding the relevance to metabolic theories of the finding that increases in gastric acid secretion are independent of a metabolic obesity. First, it will be recalled that the logic behind measuring gastric acid secretion was that it is an ideal index of vagal activity. Increased vagal tone, in turn, has been argued to be important for VMH obesity, possibly by producing overeating, metabolic

obesity, or both. While elevated vagal tone is a useful concept for explaining the effects of VMH lesions on digestive activities, it should be understood that it is the effects of VMH lesions on digestive activities that are thought to produce metabolic and behavioural adjustments favouring the development of obesity. In particular, increased insulin secretion following hypothalamic lesions has received considerable attention as a possible mechanism by which VMH lesions exert their major effects on energy stores. The present data should not be interpreted to indicate that all digestive activities, including insulin secretion must parallel the changes in acid secretion observed in PFKC and VMN rats. They may not. With respect to explaining metabolic obesity then, it remains possible that VMN lesions produce obesity via other digestive abnormalities such as hyperinsulinemia but not by a general elevation in parasympathetic responses.

A third possibility is that VMN lesions produce obesity via more subtle changes in digestive activities that are not reflected in basal secretions. For example, VMH lesions exaggerate insulin secretion to sham-feeding (Berthoud & Jeanrenaud, 1982), and post-absorptive phase insulin secretion is larger and more protracted in VMH rats (Steffens, 1970). Changes in these events could mediate the effect of VMN lesions on body fat. However, the fact that PFKC lesions produce large increases in basal secretion, but no primary adiposity, suggests that this explanation is probably incorrect.

A fourth possibility is that VMN lesions produce obesity by an impairment in growth hormone secretion. VMH lesions in weanlings lower pituitary (Reichlin, 1961) and circulating growth hormone levels (Frohman & Bernardis, 1968), and reduce skeletal growth (Frohman & Bernardis, 1968;

Han, 1967; Reichlin, 1961). In contrast, PFKC lesions demonstrated here to produce no metabolic obesity, also do not appear to stunt growth. Rather, PFKC rats lesioned as adults, have normal naso-anal lengths (Tannebaum et al., 1974). When lesioned as weanlings, knife cut rats have increased naso-anal lengths relative to brain-intact controls, suggesting that knife may increase, rather than decrease growth hormone levels (Simpson & Gold, 1981). Tannenbaum et al. (1974) have suggested that neurons originating in VMN, project to the median eminence where they stimulate the release of growth hormone releasing factor.

It is possible that impaired growth hormone secretion could produce obesity (Knobil & Hotchkiss, 1964). However, a test of this hypothesis on weanling rats has reported negative results. Goldman, Schnatz, Bernardis, & Frohman (1970) found that injections of growth hormone did not prevent the development of a primary obesity by VMH lesions in weanling rats.

Finally, it has been suggested that direct neural connections exist between VMH and adipose tissue stores. VMH lesions have long been known to produce an impairment in lipolysis. Following VMH lesions, lipolysis is impaired to a number of stimuli including; epinephrine (Haessler & Crawford, 1967; May & Beaton, 1968), or behavioural stressors such as cold, exercise, or fasting (Nishizawa & Bray, 1978). Furthermore, rats bearing VMH lesions do not display the normal daytime activation of lipolysis. Instead, nocturnal lipogenesis appears to persist (LeMagnen, et al., 1980). A direct neural effect of the VMH on adipose stores was also supported by the findings of Nishizawa and Bray (1978) described in the introduction. These researchers report that unilateral VMH lesions impair fasting induced lipogenesis from the contralateral retroperitoneal fat pad; an effect that can be mimicked by denervation of the fat pad. Finally, lipolysis is

induced by electrical stimulation of the VMH (Kumon, Hara, & Takahashi, 1976). It is possible that VMN is responsible for this influence by VMH over adipose depots.

## Summary

This thesis began with a review of the historical sequence of theories of the ventromedial hypothalamic obesity syndrome that antedated this study. These formulations were labelled "single mechanism" theories because of their emphasis on single disturbances, whether behavioural or metabolic, as major etiological factors in the development of hypothalamic obesity. Single mechanisms views are clearly contradicted by findings reported in this thesis indicating that independent mechanisms must underly the major behavioural and metabolic mechanisms of VMH obesity. Rather, these data support a dissociative perspective of the VMH syndrome.

Hyperphagia and metabolic obesity were found to be independent disturbances of lesions in the medial hypothalamus. Furthermore, vagal tone, as indexed by gastric acid secretion, was found to be independent of metabolic obesity but continued to be associated with hyperphagia (i.e. PFKC lesions). Previous research on PVH lesion rats indicates that hyperphagia and increased vagal tone are dissociable (Weingarten, et al., 1985). However, given that PVN rats are less hyperphagic than VMH rats, but PFKC rats are not, the possibility remains that peripheral physiological responses contribute to overeating. Future research should resolve this issue.

The mechanism by which VMN lesions produce obesity is also unclear. Further research should consider more specific digestive responses such as

insulin, and direct neural connections with adipose stores as possible mechanisms of VMN obesity.

Finally, other important effects of VMH lesions such as finickiness and altered affective reactions were not studied here. Therefore, the degree to which these disturbances reflect common or independent mechanisms remains an issue.

In general, these data favour a change in conceptualization of the VMH obesity syndrome and therefore a change in strategies for studying the etiology of the syndrome. These experiments suggest that it would be more profitable to investigate the specific mechanisms of hyperphagia, obesity, and altered digestive activities than to test their sufficiency as explanations of the entire syndrome.

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# APPENDICES

# APPENDIX A.

This appended manuscript is not part of the thesis proper. However, the manuscript describes research conducted by this author that is relevant to a dissociative perspective of the VMH syndrome. The experiments described in this report tested a single-mechanism theory of the ventromedial hypothalamic lesion syndrome suggested by Powley (1977) termed, "the cephalic phase hypothesis". The findings support a dissociative perspective of the VMH syndrome by: (a) providing evidence against the cephalic phase hypothesis, and, (b) demonstrating a dissociation of parasympathetic responses and VMH obesity.

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# Ventromedial Hypothalamic Lesions Eliminate Gastric Acid Secretion Elicited by Anticipated Eating

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Electrolytic lesions of the ventromedial hypothalamus (VMH) produce an obesity syndrome characterized, in part, by excessive food intake and adiposity. Several hypotheses suggest that VMH lesion-induced hyperphagia results from elevated parasympathetic tone on the viscera expressed via the vagus nerves. To evaluate this possibility, vagally-mediated gastric acid secretion was measured in control and VMH-lesion rats. Initially, Pavlovian conditioning was used to elicit acid secretion to anticipated eating. VMH lesions eliminated the ability to mobilize acid secretion to the expectation of eating even though other behavioural indices of conditioning indicated that VMH rats still expected the food. The generality of the acid secretory deficit in VMH rats was evaluated by activating vagally-mediated acid secretion pharmacologically with insulin or 2-deoxy-D-glucose (2DG). VMH rats significantly increased acid secretion to insulin, although the response was attenuated compared to controls. Acid secretion stimulated by 2DG was normal. Thus the effects of VMH lesions on vagally-mediated acid secretion depend on the way in which the response is activated. The implications of this finding are discussed.

## General Introduction

Ventromedial hypothalamic (VMH) lesions produce a variety of behavioural and metabolic disturbances including overeating (Broebeck *et al.*, 1943; Hetherington & Ranson, 1940), increased parasympathetic tone (Frohman, Frohman & Bernardis, 1968; Weingarten & Powley, 1980), and obesity (Hirsch & Han, 1969; Bernardis & Frohman, 1968). Although overeating is not required for the development of metabolic disturbances (Cox & Powley, 1981; Han, 1968; Han & Liu, 1966), a hyperphagia is a defining characteristic of the syndrome when animals are maintained *ad libitum*. The physiological disturbance mediating the overeating has not been identified. It was once proposed that the VMH controlled behavioural satiety directly and, therefore, lesions of this area enhanced eating because of a satiety dysfunction (Broebeck *et al.*, 1943; Stellar, 1954). More current hypotheses emphasize the importance of lesion-induced physiological disturbances, especially altered visceral secretion patterns mediated by the parasympathetic branch of the autonomic nervous system, to the expression of hyperphagia (Bray & York, 1979; Powley, 1977). The present experiments address these hypotheses directly by assessing the effect of VMH lesions on vagally-mediated visceral secretion.

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The autonomic nervous system, via the vagus nerves, influences both basal and stimulated visceral secretion. There is considerable information on the effects of VMH lesions on basal secretion. Hyperphagia-promoting VMH lesions are consistently associated with increases in basal gastric acid (Ridley & Brooks, 1965; Weingarten & Powley, 1980; Weingarten *et al.*, 1985) and insulin (Cox & Powley, 1981; Frohman & Bernardis, 1968) secretion, indicative of increased parasympathetic tone. Far less is known about the effects of VMH lesions on vagally-mediated stimulated digestive responses and the available data are inconsistent. For example, in contrast to the heightened basal gastric acid secretion following VMH lesions, acid secretion elicited by 2-deoxy-D-glucose (2DG) (Ridley & Cirpilli, 1969) or insulin (Ridley & Brooks, 1965) is reported to be suppressed, or eliminated, by VMH lesions. However, acid (Weingarten & Powley, 1980) and insulin (Louis-Sylvestre, 1976) secretion elicited by sham-feeding appears to be exaggerated by VMH lesions.

The purpose of the present study is to explore in detail the effects of VMH lesions on stimulated digestive secretions mediated by the vagus nerves. The digestive response used as a marker of lesion effects is gastric acid secretion. It was selected since it is a sensitive index of parasympathetic tone on the viscera, has been useful in the exposition of the effects of VMH lesions on basal secretion, and is convenient to measure. This study uses several procedures to activate vagally-mediated acid secretion including learning (i.e. anticipated eating) and pharmacological probes (insulin and 2DG).

## General Methods

### *Subjects*

Subjects were male Long Evans hooded rats approximately 90 days of age at the beginning of the experiments. Rats were bred in the McMaster Psychology colony from breeding stock obtained originally from Blue Spruce Farms (Altomont, NY). Rats were housed individually in a room maintained at 26°C and on LD 14 : 10 cycle. Water was available *ad libitum* and food was present according to the experimental protocol.

### *Surgery*

All surgery was performed while rats were anaesthetized with sodium pentobarbitol (Somnotol) injected intraperitoneally (i.p.) at a loading dose of 45 mg/kg. For gastric acid collection, rats were equipped with a chronically-indwelling stainless steel gastric cannula implanted into the forestomach. Details of cannula design and implantation surgery have been presented previously (Weingarten & Powley, 1980). Rats recovered from this surgery over a 2 week period during which they were maintained on Purina rat chow pellets. All rats also sustained VMH or sham lesions. Using standard stereotaxic procedures, and with the head positioned level, coordinates for VMH lesions were: 2·1 mm posterior to bregma, 0·6 mm lateral to the midline suture, and 8·3 mm below the skull. Bilateral electrolytic lesions were produced by passing a 1 mA anodal current for 17 sec through an electrode (#00 stainless steel insect pin insulated with epoxylite except for 0·4 mm at the tip) positioned in the VMH. The sham lesion procedure mimicked this surgery including positioning of an electrode into each VMH but no current was passed.

### *Test Cages*

Rats were tested in Plexiglas cages (10·2 cm wide × 20·3 cm long × 10·2 cm high) which had stainless steel bars as the floor. The centre bars were spaced 1·6 cm apart to permit a collection tube attached to the gastric cannula to protrude through the floor. The entire cage was mounted on 15·25 cm stilts permitting the rat to move about freely. A 2·54 cm-diameter hole was located in the centre of the cage's front wall. A graduated cylinder could be attached to the outside of the cage so that its spout was positioned in the centre of this opening. Photocells were mounted across this opening and provided an electronic signal to circuitry which recorded the amount of time rats poked their heads into the food-containing area.

### *Gastric Acid Collection and Assay*

In preparation for acid collection, each rat was removed from its home cage, its cannula was opened by removing the screw which normally kept it closed, and its stomach was cleaned by saline lavage applied through the open cannula. A 1·9 cm long stainless steel collection tube attached to a 15 cm length of Tygon tubing was threaded into the cannula. Gastric juice drained freely down this tube by gravity force and collected into a vial force fit onto the end of the Tygon tubing.

Three parameters of gastric secretion were obtained: (i) volume of secretion collected (expressed in ml); (ii) acid output (expressed as $\mu$Eq $H^+$) and determined by automatic titration (Radiometer-Copenhagen titration unit) of the sample to pH 7 using 0·05 N NaOH, and; (iii) acid concentration (expressed as $\mu$Eq $H^+$/ml) calculated by dividing the acid output by its volume.

### *Data Analysis*

Omnibus effects were analyzed by analyses of variance. Where warranted (i.e. $p < 0{\cdot}05$), multiple comparisons were performed using the Studentized range statistic ($q$) and evaluated according to the Newman–Keuls procedure.

## Experiment 1

This experiment uses a Pavlovian conditioning learning paradigm (Weingarten & Powley, 1981) to activate vagally-mediated acid secretion. Specifically, classical conditioning is used to teach an association between a specific environment and the opportunity for eating. Thus, rats learn to mobilize secretion in the anticipation of eating when exposed to that environment.

### *Protocol*

Fifty-two rats were adapted to a 17 h food deprivation schedule and divided into two groups such that the group mean body weights were equivalent. Group Condition (COND) rats were conditioned to associate the test cage with the availability of food. This was accomplished by placing the animals into the test cages and, 30 min later, providing them with an opportunity to feed an evaporated milk-based liquid diet for

20 min. Gastric secretion was collected during the 30 min period prior to food presentation to obtain an index of anticipatory acid secretion. Group Non-Condition (NON-COND) animals were treated similarly except that no food was presented to them at the end of the 30 min. All rats were returned to the home cages after these daily trials and were provided with a food ration sufficient to maintain the deprivation schedule. This training continued for 12 consecutive days. On the 13th day, two-thirds of the rats in each group sustained VMH lesions (VMH) and the remaining third underwent the sham lesion (SHAM) procedure. This produced a total of four groups differing in their association of the test environment with food (COND versus NON-COND) and brain surgery (VMH vs. SHAM). Animals were assigned to the VMH or SHAM conditions based upon their acid outputs on the last 3 days of testing such that the average acid outputs of the two COND groups would be similar. To eliminate problems associated with feeding rats immediately following VMH lesions, all rats were deprived of food for 24 h after stereotaxic surgery. On day 2 postlesion, rats were tested daily in the manner described for prelesion testing. This testing continued for 8 consecutive days. To ensure that VMH rats did not gain more weight than controls during this period, the amount of food provided daily to individual lesion animals was controlled to maintain the weights of VMH animals equivalent to those of SHAM animals matched for weight at the time of surgery.

At the completion of testing, all rats were placed *ad libitum* on a high fat diet (66% Purina powder, 33% Crisco oil, by weight) for 3 weeks to obtain a functional index of the VMH lesions. Then, rats were sacrificed and perfused intracardially with saline and 10% buffered formalin. The brains were removed, frozen, sectioned at 40 $\mu$, and stained with cresyl violet and luxol fast blue for histological examination.

## *Results*

### *Histology*

The extent of neural damage was assessed by an investigator blind to the experimental results. The selection of animals into the two VMH groups was based upon a histological criterion which selected rats with almost-to-complete bilateral destruction of the classically-defined ventromedial hypothalamus (Weingarten & Powley, 1980). Based upon this criterion, 15 of the original 36 rats which had sustained lesions were included in the eventual VMH groups. Eight were in the COND/VMH group and 7 in the NON-COND/VMH group. Results describing the effects of VMH lesions are based upon the performance of these 15 animals.

### *Body weight*

Figure 1 shows the body weights of the groups at the various phases of the experiment. Analyses of variance indicated the body weights of the four groups did not differ significantly at surgery, $F(3,30)=0{\cdot}71$, $p>0{\cdot}05$, nor on the first, $F(3,30)=0{\cdot}54$, $p>0{\cdot}05$, or last, $F(3,30)=0{\cdot}32$, $p>0{\cdot}05$, day of prelesion conditioning. Most importantly, the restricted feeding schedules imposed postlesion were successful in maintaining the weights of VMH rats at control levels throughout the postlesion acid sampling period, as indicated by the absence of any group weight differences on the 8 postlesion days, $F(3,27)=1{\cdot}34$, $p>0{\cdot}05$.

The histological criterion used to select VMH animals did identify rats which were hyperphagic. After 3-weeks *ad libitum* on a high fat diet, groups differed significantly in

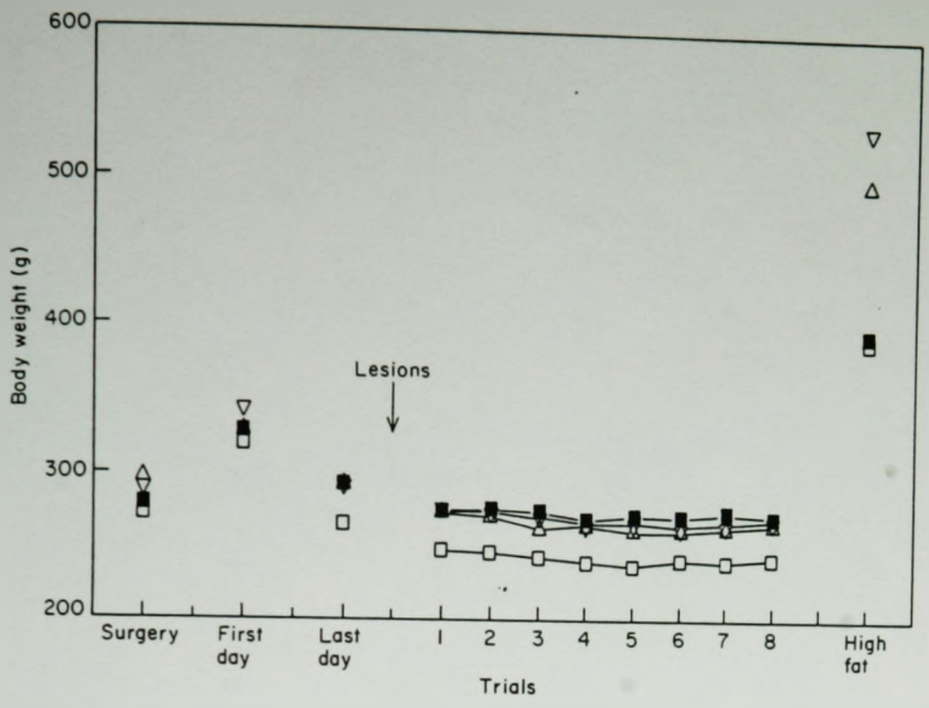


FIGURE 1. Group mean body weights during various phases of Experiment 1. Surgery refers to weights at time of gastric cannula surgery. First day and Last day refer to the first and last days of prelesion conditioning. Trials 1–8 indicate weights on each day of postlesion testing. High fat refers to weights after 3 weeks *ad libitum* high fat diet. Note that all rats maintained similar weights throughout the prelesion and postlesion acid sampling periods but that both VMH groups were hyperphagic and became obese on high fat. (□, SL-NCTN = sham lesion, noncondition rats, $N = 8$; ■. SL-CTN = sham lesion, condition rats, $N = 8$; △, VM-NCTN = ventromedial lesions. noncondition rats, $N = 7$; ▽, VM-CTN = ventromedial lesions, condition rats, $N = 8$).

weight, $F(3,25) = 18{\cdot}43$, $p < 0{\cdot}01$. Multiple comparisons revealed that both VMH groups weighed significantly more than controls ($p < 0{\cdot}01$) but that the final weight of the two VMH groups did not differ significantly from one another ($p > 0{\cdot}05$).

*Gastric secretion*

Secretion data, shown in Figure 2, were analyzed with analysis of variance. A separate ANOVA was performed for each of the three parameters of secretion.

On the first day of conditioning, the four groups had similar volumes, $F(3,27) = 1{\cdot}28$, $p > 0{\cdot}05$, outputs, $F(3,27) = 1{\cdot}34$, $p > 0{\cdot}05$ and concentrations. $F(3,27) = 0{\cdot}74$, $p > 0{\cdot}05$, of secretion. The conditioning procedure, however, was effective in developing an anticipatory gastric secretory response. Analysis of secretion on the last three 2-day blocks prelesion indicated that COND rats secreted more volume, $F(1,27) = 13{\cdot}73$, $p < 0{\cdot}01$; and acid $F(1,27) = 48{\cdot}18$, $p < 0{\cdot}01$ than NON-COND animals. Their gastric juice also had a significantly higher acid concentration. $F(1,27) = 45{\cdot}41$ $p < 0{\cdot}001$. Furthermore, the acid secretory responses at this point were stable as the analysis of variance revealed no significant changes in the magnitudes of response over the 6 days (all days effects were $p > 0{\cdot}05$). In addition, although the overall experience of conditioning was clearly evident on these final prelesion days, rats to be subject to VMH lesions were not different at this point from their respective SHAM controls.

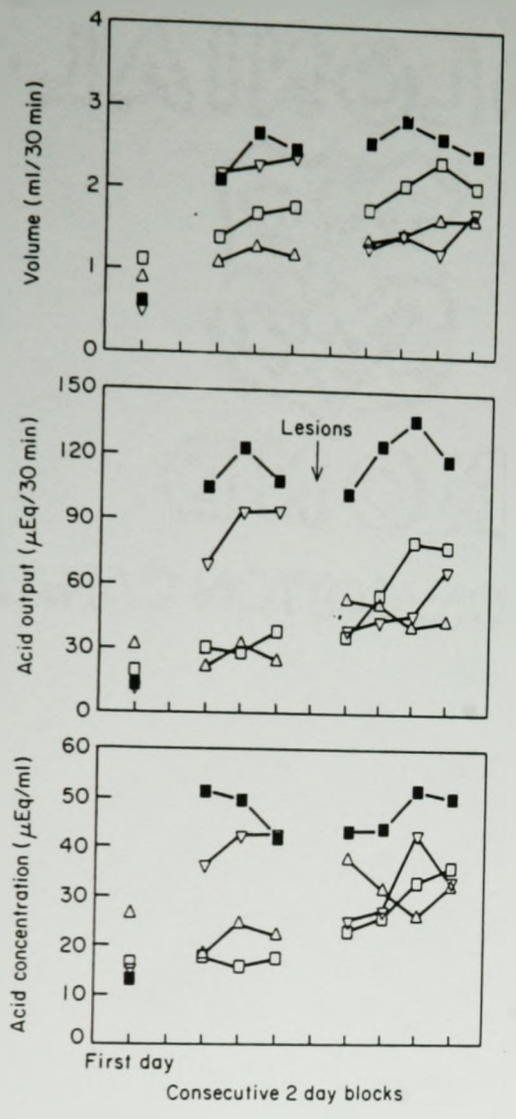


FIGURE 2. Group mean acid secretions in Experiment 1. First day indicates levels of secretion on the first test day. The following three data points indicate responses for the final six prelesion days organized as 2-day blocks. The last four data points indicate postlesion levels of response organized as two-day blocks. Abbreviations and group sizes as Figure 1.

The major effect of VMH lesions was to eliminate the anticipatory acid response. The loss of anticipatory responding in VMH rats, but its persistence in SHAM animals, resulted in a significant Lesion × Condition interaction for acid output $F(1,27) = 7{\cdot}41$, $p < 0{\cdot}01$ and concentration $F(1,27) = 4{\cdot}05$, $p < 0{\cdot}05$. This interaction did not reach statistical significance for volume $F(1,27) = 1{\cdot}82$, $p > 0{\cdot}05$. To locate the source of this interaction on output and concentration data, separate $F$ tests were performed within each factor (Lesion and Condition) to permit the following comparisons: SHAM/NON-COND vs. VMH/NON-COND, SHAM/COND vs. VMH/COND, SHAM/NON-COND vs. SHAM/COND and VMH/NON-COND vs. VMH/COND. The absence of a conditioned acid secretion in VMH rats was indicated by two results of these tests. First, VMH-COND rats demonstrated less acid output $F(1,27) = 25{\cdot}17$, $p < 0{\cdot}001$ and acid concentration $F(1,27) = 6{\cdot}28$, $p < 0{\cdot}05$ than SHAM/COND rats. Second, while SHAM/COND rats maintained elevated acid output $F(1,27) = 16{\cdot}53$,

$p<0{\cdot}001$, and concentration $F(1,27)=8{\cdot}77$, $p<0{\cdot}01$ relative to SHAM/NON-COND rats, VMH/COND rats were not different from VMH/NON-COND rats in either output $F(1,27)=0{\cdot}02$, $p>0{\cdot}05$ or concentration $F(1,27)=0{\cdot}01$, $p>0{\cdot}05$.

The elimination of anticipatory secretion as a result of VMH lesions is shown more directly in Figure 3 which presents the prelesion to postlesion difference in secretion for each of the four groups. Analyses of variance revealed that the groups differed significantly in pre- to postlesion changes of volume, $F(3,27)=5{\cdot}064$, $p<0{\cdot}01$; acid output, $F(3,27)=8{\cdot}69$, $p<0{\cdot}001$; and acid concentration $F(3,27)=6{\cdot}13$, $p<0{\cdot}01$. *Post-hoc* tests revealed that this significant Group finding resulted from the fact that VMH/COND rats had a significantly greater pre- to postlesion difference in secretion than all other groups; this resulted from a significant decline in secretion postlesion. This change was evident in the volume ($p<0{\cdot}05$), acid output ($p<0{\cdot}01$), and acid concentration ($p<0{\cdot}01$) measures of secretion.

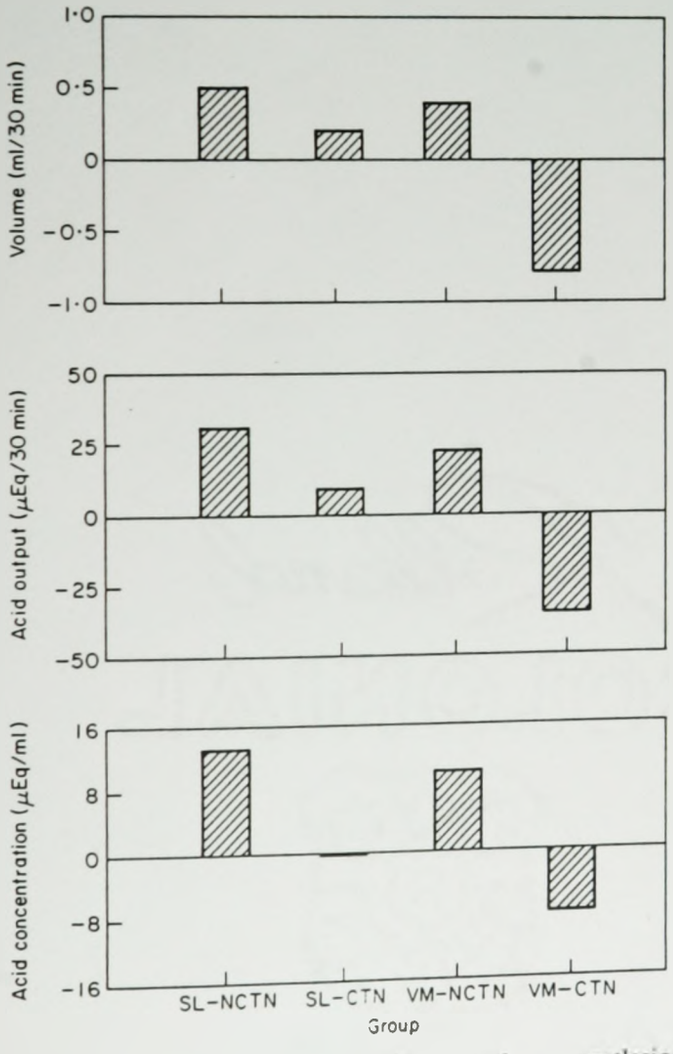


FIGURE 3. Group mean differences in secretion prelesion to postlesion. For each animal, a difference score was generated by subtracting average secretion level on Days 1–8 postlesion from average secretion level on the last three prelesion blocks. Abbreviations and group sizes as Figure 1.

*Nose-poking data*

The elimination of anticipatory acid secretion did not result from forgetting the association between the test environment and food. Table 1 displays the time that animals nose-poked into the food cup 5 min prior to the delivery of food (when the majority of nose-poking occurred). Prelesion, SHAM/COND rats nose-poked more than SHAM/NON-COND rats, $t(14)=2{\cdot}14$, $p<0{\cdot}05$, and VMH/COND rats nose-poked more than VMH/NON-COND rats, $t(13)=3{\cdot}32$, $p<0{\cdot}01$. The amount of nose-poking in the two COND groups was similar. Postlesion, both SHAM/COND rats $t(14)=3{\cdot}26$, $p<0{\cdot}01$ and VMH/COND rats $t(13)=3{\cdot}35$, $p<0{\cdot}01$ maintained elevated anticipatory nose-poking relative to respective controls. Further, the two COND groups were still not significantly different from one another in nose-poking activity.

### *Discussion*

This experiment replicates the observation that anticipated eating is a reliable and potent elicitor of acid secretion in the rat (Weingarten & Powley, 1981). In this study, using this learning procedure, a threefold increase in secretion is obtained within five to seven environment-food pairings. The major finding of the present experiment, however, is that VMH lesions eliminate acid secretion elicited by anticipated eating. This result is in direct contrast to the prediction of Powley's (1977) cephalic phase hypothesis which suggests that VMH lesions should result in an exaggeration of anticipatory acid secretion. The failure of VMH rats to mobilize acid secretion to the expectation of eating cannot be attributed to a memory disturbance since a behavioural index of conditioning, nose-poking, reveals that VMH rats continued to anticipate the meal in that environment.

## Experiment 2

The previous experiment demonstrated a disruption of vagally-mediated acid secretion elicited by conditioning after VMH lesion. The present experiment assesses

Table 1

*Mean number of seconds spent nose-poking at the locus of food presentation 5 minutes prior to food delivery*

| Group | Prelesion | Postlesion |
|---|---|---|
| SHAM/NON-COND | 3·7 (1·6) | 5·8 (2·6) |
| SHAM/COND | 22·5 (8·7) | 32·6 (7·8) |
| VMH/NON-COND | 3·7 (1·5) | 1·0 (0·5) |
| VMH/COND | 19·2 (4·2) | 29·7 (8·0) |

*Note*: Means represent the group average over the last four trials prelesion or postlesion. Figures in brackets represent 1 *SEM*.

the generality of this finding by examining the effects of VMH lesions on acid responses which are also expressed via the vagus nerves but which are elicited pharmacologically by administration of insulin (LaBarre & deCespedes, 1931) or 2DG (Duke *et al.*, 1970).

### *Method*

Thirty rats were implanted with gastric cannulae and were permitted to recover for 2 weeks. Nineteen subjects sustained bilateral VMH lesions according to the procedures described before; the remaining 11 rats received sham lesions.

Acid collections were conducted three times per week and were 2 h in duration. Rats were 17 h food deprived prior to each test. Basal secretion was monitored for five test sessions before the administration of any drugs. To examine the response to insulin, regular insulin (Connaught) in doses of 0·25, 0·50 and 0·75 U/kg were injected subcutaneously (s.c.) 30 min prior to the initiation of a 2 h acid collection period. For control trials, 0·9% saline was injected instead of insulin and these were interposed once every three trials. The order of insulin dose administrations was counterbalanced across trials. 2DG (Sigma, St Louis, MO) was injected s.c. at a dose of 100 mg/kg 30 min prior to initiation of acid collections. After acid testing, subjects were returned to the colony room and food was made available one hour later. As in Experiment 1, body weights of lesion animals were maintained at control levels by restricted feeding.

At the end of the experiment, animals were sacrificed with 1·0 ml of 50% chloral hydrate. The brains of VMH rats were removed, and animals were included into the VMH group based upon an assessment of the locus and extent of VMH damage according to the procedures described in Experiment 1. Six rats were included in the final VMH group.

### *Results*

Between groups *t*-tests on secretion during the five basal sessions prior to the initiation of drug administration indicated that VMH lesions produced an elevation in volume, $t(13) = 2{\cdot}94$, $p < 0{\cdot}05$, and acid output, $t(13) = 2{\cdot}60$, $p < 0{\cdot}05$. Acid concentration was also elevated in VMH rats but the group difference did not attain statistical significance, $t(13) = 0{\cdot}21$, $p > 0{\cdot}05$. These values are provided in Table 2.

In general, insulin increased acid secretion. Examination of Figure 4 and statistical analyses revealed, however, that the effects of increasing doses of insulin on gastric

TABLE 2
*Effects of VMH lesions on basal gastric acid secretion*

| | SHAM ($N = 9$) | VMH ($N = 6$) |
|---|---|---|
| Volume (ml) | 2·04 ± 0·4 | 3·92 ± 0·5* |
| Acid output ($\mu$Eq) | 89·4 ± 21·0 | 178·5 ± 27·9* |
| Acid concentration ($\mu$Eq/ml) | 40·8 ± 8·3 | 46·7 ± 7·1 |

*Note*: Values represent $\bar{X} \pm 1$ *SEM*. (* indicates sham vs. VMH comparison is $p < 0{\cdot}05$.)

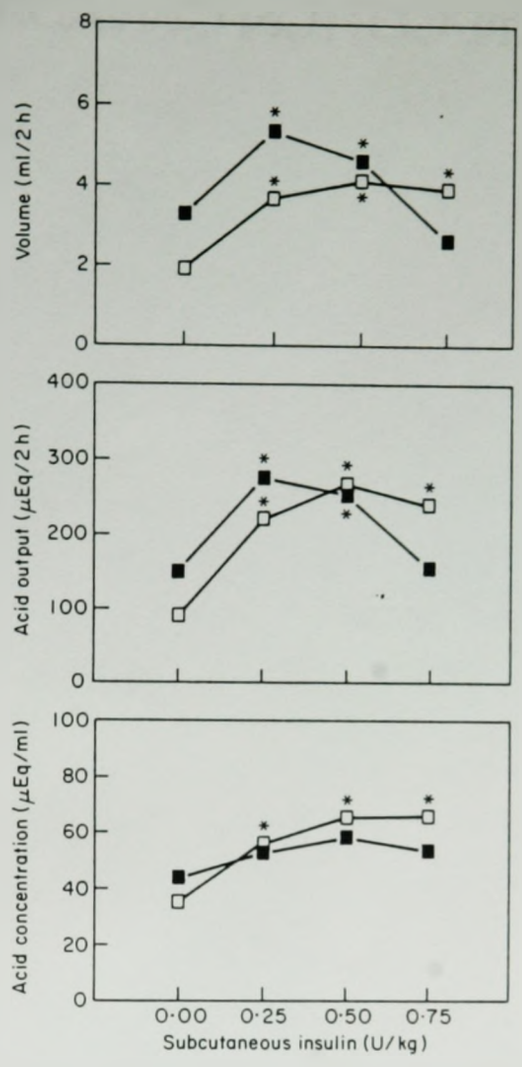


FIGURE 4. Group mean secretion values for □, sham ($N=9$) and ■, VMH lesion ($N=6$) rats to s.c. insulin at doses indicated. Asterisks represent significant elevation over saline value within that group.

secretion differed between the two groups. This was evidenced by a significant Group X Dose interaction for volume, $F(3,39)=3{\cdot}94$, $p<0{\cdot}05$ and acid output, $F(3,33)=2{\cdot}84$, $p<0{\cdot}05$. Multiple comparisons to identify the source of the interaction revealed that SHAM rats significantly increased volume, ($p<0{\cdot}05$) output ($p<0{\cdot}01$) and concentration ($p<0{\cdot}05$) of secretion at all three insulin doses compared to saline control trials. In contrast, VMH rats significantly increased volume and acid output at both 0·25 and 0·50 U/kg ($p<0{\cdot}01$), but not at 0·75 U/kg. Further, acid concentration was not significantly elevated by any dose of insulin in VMH rats.

The acid responses to 2DG are displayed in Figure 5. (One animal in the Sham group was excluded from the data analysis because the cannula did not remain patent.) VMH and SHAM rats had comparable responses to 2DG. SHAM rats demonstrated significant elevations in acid output, $t(7)=2{\cdot}49$, $p<0{\cdot}05$, and concentration, $t(7)=2{\cdot}49$, $p<0{\cdot}05$, but not volume, $t(7)=1{\cdot}36$, $p>0{\cdot}05$ to 100 mg/kg 2DG. Similarly, VMH rats

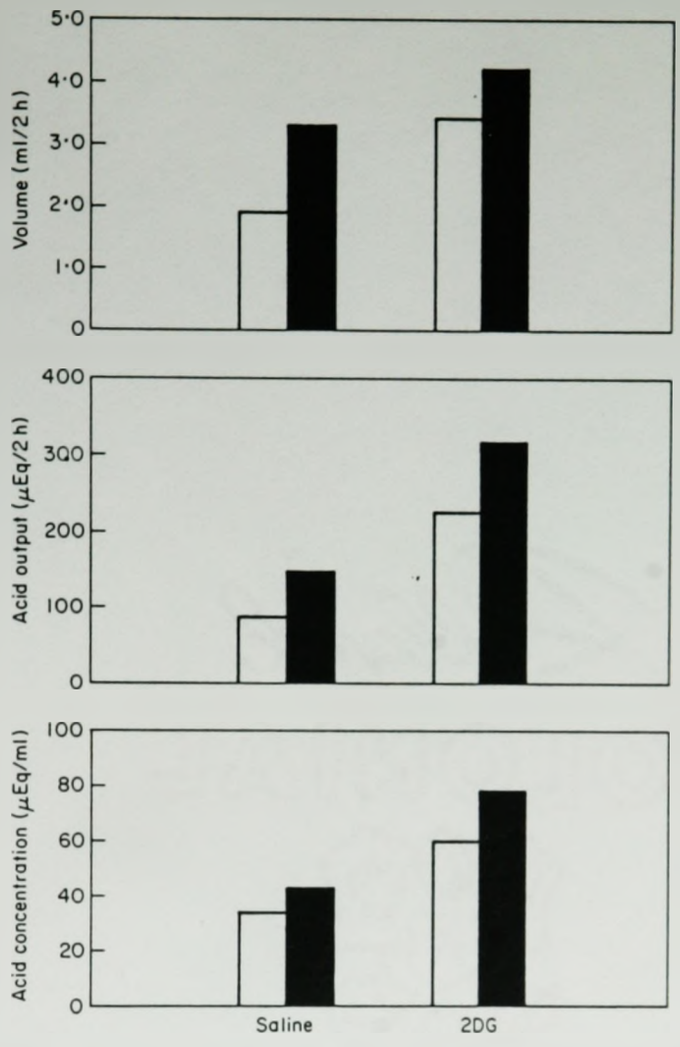


FIGURE 5. Group mean secretion values for □, sham ($N = 9$) and ■, VMH lesion ($N = 8$) rats in response to injections of 100 mg/kg 2-deoxy-D-glucose or equivolumes of 0·9% saline.

also demonstrated significant elevations of acid output, $t(5) = 3{\cdot}50$, $p > 0{\cdot}05$, and concentration, $t(5) = 8{\cdot}29$, $p < 0{\cdot}001$. Volume was not significantly altered, $t(5) = 1{\cdot}10$, $p > 0{\cdot}05$ to this dose of 2DG.

## *Discussion*

VMH lesions do not eliminate gastric acid secretion to insulin or 2DG-induced glucoprivation. There are some differences between VMH and control rats in response to insulin. Controls increase secretion to the whole range of insulin doses used (0·25 to 0·75 U/kg). Overall, VMH rats mobilize an attenuated response relative to controls. However, the degree of attenuation depended on dose as VMH rats increase secretion to low, but not high, doses of insulin. These data replicate the report of Ridley and Brooks (1965) that VMH rats fail to hypersecrete to high dose insulin. However, the present results extend that finding by demonstrating that this does not reflect a generalized inability of VMH rats to respond to insulin but rather that a lesion by dose

interaction is apparent. VMH rats, like controls, increased secretion to 2DG. This finding contrasts with Ridley and Cirpilli (1969) who reported an elimination of 2DG-elicited acid secretion in VMH rats. However, studies which have isolated the glucose-sensitive cells responsible for the 2DG-induced acid reflex in the lateral hypothalamus (Colin-Jones & Himsworth, 1970; Kadekaro *et al.*, 1977) may explain these discordant observations. In our studies, rats were selected which had an extensive VMH damage but which sustained no damage to the lateral hypothalamus and, therefore, cells which mediate 2DG-elicited acid secretion. In contrast, the lesions of Ridley and Cirpilli (1969) are more extensive and appear to invade lateral hypothalamic cell bodies.

## General Discussion

The results of these experiments indicate that the effects of VMH lesions on vagally-mediated gastric acid secretion depend on the stimulus used to activate the response. VMH lesions eliminate responding to anticipated eating, attenuate responding to insulin-induced secretion, and have no impact on 2DG induced secretion. The finding of an elimination of anticipatory acid secretion by VMH lesions is consistent with the report that VMH rats also fail to mobilize insulin secretion in the expectation of eating (Storlien, 1985).

The elimination of anticipatory acid secretion in VMH rats cannot reflect a lesion-induced forgetting of the association between the environment and food since another measure of this association, nose-poking into the food cup prior to food delivery, provides no evidence that the VMH rats have forgotten the relationship between the test environment and food. The persistence of an elevated behavioural index of conditioning, nose-poking, in the face of an eliminated visceral index, acid secretion, suggests that VMH rats have lost the ability to mobilize specifically the visceral response. This conclusion is reinforced by a trial-by-trial analysis of the conditioning preparation used here. The magnitude of conditioned anticipatory acid secretion asymptotes by about the fifth environment-food pairing (see also Weingarten & Powley, 1981). In the present study, rats received eight trials postlesion. Even if VMH lesions resulted in the forgetting of the environment-food association, there are sufficient postlesion conditioning trials for them to re-acquire the association, and no such tendency is observed.

It is also unlikely that the failure to demonstrate an anticipatory acid response reflects some non-specific interaction between the test situation and VMH lesions. It is unlikely that the handling procedures preliminary to acid collection engenders excessive stress in VMH animals thereby suppressing anticipatory acid secretion. In the literature, stress is as often associated with increases, as with decreases, of acid secretion (Brodie *et al.*, 1962; Ludwig & Lipkin, 1969; Menguy, 1960). A study designed specifically to evaluate the effect of handling stress demonstrated that handling increased acid secretion (Weingarten, Note 1). Finally, in a series of unpublished studies, we verified that stressors such as excessive handling and foot shock resulted in no (or, at most, a very transient and small) reduction in an established conditioned anticipatory acid response.

The inability of VMH rats to mobilize an anticipatory acid response does not reflect a general impairment in their ability to elicit vagally-mediated secretion since they can mobilize acid to insulin- and 2DG-induced hypoglycaemia. The subdiaphragmatic

vagus nerves represent the final common path for the expression of anticipatory-, insulin- and 2DG-stimulated acid secretion, as well as for sham feeding-elicited secretion (Farrell, 1928). The specific loss of anticipatory acid secretion conditioned to food, but the preservation of these other vagal responses, after VMH lesions indicates this brain site as critical to the elaboration of this food-associated response but not to these other brain-elicited secretions. Brain receptors mediating insulin- and 2DG-induced acid secretion have been identified in the hindbrain and hypothalamic areas other than the VMH (Colin-Jones & Himsworth, 1970; Kadekaro *et al.*, 1977). Currently, the term "cephalic" is often used to designate digestive responses mediated by a brain receptor (e.g. Konturek, 1974). The fact that VMH lesions can differentially affect cephalic responses depending on the stimulus used to evoke them suggests that any general statement regarding function or consequence of the entire class of cephalic phase secretions must be interpreted with caution unless a range of cephalic phase response are examined.

Many commentators, motivated by the belief that visceral secretions drive feeding, have hypothesized that excessive eating is associated with elevated anticipatory secretion (Berthoud *et al.*, 1981; Geiselman & Novin, 1982; Nicolaïdis, 1977; Powley, 1977; Rodin, 1985). In fact, analysis of the relationship between disturbances of visceral secretion and eating in the VMH rat helped fuel such hypotheses. This contention, however, has received little experimental support. There is little convincing data to support the idea that the brain detects the level of visceral secretion and modulates the degree of eating in light of the magnitude of that signal. Elimination of anticipatory peripheral secretion pharmacologically with cholinergic blockers has no impact on the amount taken at a mean (Weingarten, 1984) and it appears that anticipatory visceral responses are not even activated proximal or coincident with meal initiation (Berthoud & Powley, 1986). The fact that VMH lesions lead to the elimination of anticipatory acid (present study) and insulin secretion (Storlien, 1985) suggests that it is as reasonable to investigate the correlation between decreases of anticipatory secretion and overeating as the correlation between increase in these responses and hyperphagia.

Anticipatory digestive responses have a well-documented physiological function in optimizing the process of digestion (Molina *et al.*, 1977; Nicolaïdis, 1977; Pavlov, 1910). The absence of anticipatory digestive response may contribute to VMH-induced obesity. For example, Storlien (1985) has suggested that loss of early insulin release may cause the exaggerated and protracted insulin release characteristic of postprandial secretion in VMH rats (Steffens, 1970). Also, chronic intragastric feeding of rats, thus bypassing anticipatory- and oropharyngeal- related secretion, results in excess adiposity relative to oral consumption of an identical number of calories ingested in an identical meal pattern (Cox & Powley, 1981). Thus, some of the excess adiposity in the *ad libitum* and even the pair-fed VMH rat may result from the loss of anticipatory secretion. Resolution of this issue requires more extensive investigation of the function of anticipatory secretion in digestion and the significance of its loss.

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## APPENDIX B.

### COMPARISON OF CONTROL GROUP MEANS ON DEPENDENT MEASURES

| MEASURE | CONTROL GROUP | |
|---|---|---|
| | Experiment 1a | |
| | SHAM-L | SHAM-KC |
| Wt. Gain (g) | 208.4 (15.4) | 177.0 (14.7) |
| 24 hr Kcal | 117.2 (3.5) | 112.6 (2.7) |
| % Fat | 21.9 (1.7) | 23.8 (1.5) |
| | Experiment 1b | |
| | CONT | SHAM (L+KC) |
| Wt. Gain (g) | 144.0 (22.3) | 148.6 (9.9) |
| 24 hr Kcal | 106.5 (3.2) | 110.8 (1.4) |

Experiment 2

| | SHAM-L | SHAM-KC |
|---|---|---|
| Wt. Gain (g) | 126.2<br>(8.5) | 128.7<br>(14.5) |
| % Fat | 13.6<br>(1.3) | 12.3<br>(1.3) |
| Pre-Post Lesion<br>Gastric Secretion Changes | | |
| Volume (ml/2hr) | -0.03<br>(0.33) | 0.47<br>(0.46) |
| Acid Output<br>(uEq/2hr) | 32.6<br>(20.3) | 90.2<br>(29.5) |
| Acid Concentration<br>(uEq/ml) | 16.6<br>(5.8) | 26.9<br>(5.7) |

Numbers in parentheses are 1 standard error of the mean.

Control groups are not significantly different on any measure.