

THE EFFECTS OF CHRONIC ELECTRICAL STIMULATION OF THE  
VENTROMEDIAL HYPOTHALAMIC NUCLEUS IN THE RAT

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A thesis submitted to the School of Graduate Studies and  
Research of the University of Ottawa as partial fulfilment  
of the requirements for the degree of Doctor of Philosophy



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"Our minds possess by nature an insatiable desire  
to know the truth"

Marcus Tullius Cicero

(106-43 B.C.)

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### Abstract

Despite the plethora of studies documenting the effects of ventromedial hypothalamic (VMH) stimulation, very little of this work has focused specifically on the metabolic consequences of chronic activation of this structure. Hence, the purpose of this thesis was to examine more closely the changes in body growth following repeated exposure to VMH stimulation. In the first experiment, body weight, food intake, and epididymal fat pad weight were evaluated in three groups of rats receiving either VMH stimulation, no stimulation (implanted control), or stimulation to areas adjacent to the VMH. Three hours of intermittent low level electrical stimulation were delivered three times per week for four weeks, after which the animals were monitored for another 10 days. There was a significant difference in the rate of weight gain between the VMH stimulated and the implanted control groups. The efficiency of food utilization was dramatically less for the first week of stimulation in the VMH group as compared to either of the control groups. There was no significant difference among groups in the amount of food eaten nor in their epididymal fat pad weights. These data indicate that chronic low level VMH stimulation results in a significantly reduced weight gain, but that the non-specific effects of electrical brain stimulation account for approximately 50% of this reduction.

In the second experiment rats received only one week of stimulation (or three stimulation sessions) in order to determine if relatively little stimulation could produce a chronic, that is at least a four week, reduction in body weight. The contribution of stimulation-induced activity and brown adipose tissue thermogenesis to this effect was also examined. Consistent with the results of the first experiment, no statistically significant difference in the rate of weight gain was observed between the VMH and extra-VMH groups, although visual examination of the data shows that VMH stimulation gives rise to a shallower growth curve. Stimulation bound activity, which generally was associated with VMH sites, was the most important contributing factor to a reduction in weight gain. As a group, rats showing high levels of stimulation bound activity also showed a significant depression of food intake during the week of stimulation. These results emphasize the importance of dissociating the contribution of stimulation-induced activity from that of the other effects of electrical brain stimulation. Only two sites, one VMH and one extra-VMH, were accompanied by an increase in brown adipose tissue thermogenesis when core body temperature was maintained with a homeothermic blanket unit during stimulation.

The data from the rats in both experiments were pooled and evaluated using a multidimensional scaling (MDS) analysis. The two dimensional MDS solution showed that rats

with electrode placements within the VMH tended to gain less weight and eat less food than rats with electrode placements at similar anteroposterior coordinates, but outside the VMH.

An increased lipolysis and/or decreased lipogenesis may contribute to the inhibition of weight gain observed in rats that receive VMH stimulation. A technology, such as the SA-2 small research animal body composition analyzer, that permits the monitoring of body composition over time would clarify this issue. The third experiment was designed to evaluate the reliability and validity of this technique. It appears that serial estimates of body composition may be problematic and that at the moment, there is no useful non-invasive technique for tracking body composition in chronic stimulation studies.

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## INTRODUCTION

The survival of the species is dependent on certain behaviours or patterns of behaviours such as those associated with feeding, drinking, procreation, and self-defense. The central role of the ventromedial nucleus of the hypothalamus (VMH) in the mediation of these activities has been recognized for more than half a century as demonstrated by the work of Hetherington and Ranson (1939, 1940a, 1940b). More contemporary studies have confirmed that the VMH is a key player in the regulation of metabolic homeostasis and food intake (for reviews see Frohman, 1980; Powley, Opsahl, Cox, & Weingarten, 1980). Yet, despite years of work, the exact mechanisms whereby this nucleus produces its central and peripheral effects are not fully understood. This is hardly surprising, given both the functional and anatomical complexity of the hypothalamic region as a whole (see Berk & Finkelstein, 1981; Krieger, Conrad, & Pfaff, 1979; Frohman, 1980; Luiten, Ter Horst, & Steffens, 1987; Powley, Opsahl, Cox, & Weingarten, 1980; Saper, Swanson, & Cowan, 1976).

Both lesion and stimulation paradigms have been used to examine the importance of the VMH in the control of various aspects of energy metabolism. The former grew out of the observation that abnormal obesity in both humans and animals was associated with lesions at the base of the diencephalon

(Hetherington & Ranson, 1940); this marked the beginning of the inquiry concerning the role of the VMH in metabolic homeostasis. An overview of this work is provided in later sections of the Introduction.

### **Ventromedial Hypothalamic Pathways**

In mammals, the hypothalamus is important in both the integration and the relay of information to and from the organism (Luiten et al., 1987). Not unexpected given its rich functional significance, the anatomy of this relatively small brain region is very complicated. This complexity is apparent when considering the circuitry of the VMH, in particular. For example, it receives input from two dominant limbic structures, the amygdaloid body and hippocampal formation, as well as from ascending pathways that carry viscerosensory information (Luiten et al., 1987; Ricardo, 1983). With respect to intrahypothalamic connections, its output is largely confined to the periventricular region and the dorsomedial nucleus (Luiten et al., 1987). The following sections provide more detailed descriptions of the major input and outflow pathways to and from the VMH, and elaborate on its intrinsic connections within the hypothalamic area.

### VMH Afferents

Retrograde and anterograde transport labelling methods have shown that the VMH receives considerable input from the limbic system (Krettek & Price, 1978; Kita & Oomura, 1982; Luiten et al., 1987; Luiten, Ono, Nishijo, & Fukuda, 1983; McBride & Sutin, 1977; Ricardo, 1983). For example, Luiten et al. (1987) used a retrograde tracer to demonstrate that a substantial portion of VMH input consists of descending afferent projections. At their most rostral level, cells within the lateral septal nuclei picked up the marker; more caudally, labelling appeared in the bed nucleus of the stria terminalis, the supraoptic nucleus, and the medial preoptic area (Luiten & Room, 1980; Luiten et al., 1987). Continuing in a posterior direction to the level of the anterior hypothalamus, they found that the VMH receives afferent projections from the parvocellular part of the paraventricular nucleus, the anterior hypothalamic nucleus, and the medial and lateral aspects of the retrochiasmatic area, which is located at the base of hypothalamus (Luiten & Room, 1980; Luiten et al., 1987). At the level of the injection site, retrograde labelling was seen in the dorsomedial hypothalamic nucleus and the amygdaloid body; with respect to the latter, the medial amygdaloid nucleus was the most densely labelled. At a more posterior level, labelling was present in the amygdalohippocampal area and the ventral subiculum of the hippocampal formation (Luiten

et al., 1987). The specific projections of the amygdala to the VMH have been examined in detail in a number of studies (Krettek & Price, 1978; Luiten, Ono, Nishijo, & Fukuda, 1983; Luiten et al., 1987). Using anterograde tracing methods Krettek and Price (1978) identified two amygdaloid fiber systems which terminate in the VMH. One system became labelled following injections into the medial and basomedial amygdaloid nuclei and it projects to the cellular core of the nucleus; the other, which terminates mainly in the cell sparse capsule that surrounds the VMH (Millhouse, 1973a, 1973b), becomes labelled after injections into the ventral part of the subiculum, the amygdalo-hippocampal area, and possibly the posterior cortical nucleus (Krettek & Price, 1978).

The results from work using retrograde tracing methods (Berk & Finkelstein, 1981) are consistent with these findings. More recent studies emphasize that the VMH as a whole receives projections mainly from the medial amygdaloid nucleus via the stria terminalis (Luiten et al., 1983; Luiten et al., 1987). Specifically, Luiten et al. (1983) found that the anterior portion of the medial amygdaloid nucleus has extensive projections to anterior and posterior dorsal subdivisions of the VMH, whereas more posterior aspects of this nucleus project to the ventral region of the VMH. To a lesser degree, the VMH also receives input from the central, basolateral, basomedial, and lateroposterior

nuclei, and the area around the intercalated mass (Berk & Finkelstein, 1981; Luiten et al., 1983). However, less significance seems to be attached to these latter projections - presumably due to their relative sparsity when compared to efferents from the medial amygdaloid nucleus. Hence, the major amygdaloid input to the VMH consists of descending fibers from the medial nucleus that run in a medial periventricular position and then curve ventrolaterally around the VMH; they terminate both within the capsule of fibers that surrounds the nucleus and within the ventrolateral core of the VMH itself (Luiten et al., 1987).

According to Luiten et al. (1987), a considerable portion of afferent input to the VMH has its origin in the amygdaloid body and the hippocampal formation, and they suggest that information transfer to the nucleus from these two structures occurs by both direct and indirect connections. Specifically, the hippocampal formation has direct access to the VMH by way of the ventral subiculum; however, it is also proposed that the VMH receives input from the hippocampal formation indirectly via the lateral septum (Luiten et al., 1987). Similarly, the VMH probably receives input from the amygdala directly by way of the amygdalofugal pathway and indirectly via the bed nucleus of the stria terminalis; although in the latter case, there are no projections to the core of the VMH (Krettek & Price, 1978; Luiten et al., 1987).

In the mesencephalon, Berk and Finkelstein (1981) report some retrograde labelling in both the periaqueductal grey region and the raphe nuclei; a much heavier concentration of label was observed in the peripeduncular nucleus. In addition, their results showed that the VMH receives afferent projections from the pons, specifically the locus coeruleus, dorsolateral tegmental area, nucleus incertus, and the lateral parabrachial nucleus. The latter had a very high concentration of label suggesting that it is a rich source of input to the VMH (Berk & Finkelstein, 1981). The VMH is also the recipient of both direct and indirect input from the caudal part of the solitary nucleus and tract (Berk & Finkelstein, 1981; Ricardo, 1983). Other afferent projections to the VMH arise in the area adjacent to the medial longitudinal fasciculus and the lateral reticular nucleus (Berk & Finkelstein, 1981). Kita and Oomura (1982) report VMH input from the peripeduncular nucleus and an area lateral to the brachium conjunctivum at the level of the pons. McBride and Sutin (1977) describe afferent projections to the VMH (in the cat) from the region just lateral to, or dorsolateral to, the brachium conjunctive and, consistent with previous work done in rats, they also observed labelled cells in the lateral parabrachial nucleus. Hence, it would appear that there is an important ascending (as well as descending) component to VMH afferents.

### VMH Efferents

Generally speaking, many of the efferent and afferent connections of the hypothalamus are reciprocal in nature (Ricardo, 1983). There appears to have been only two studies that have specifically examined the efferent connections of the VMH (see Krieger et al., 1979; Saper, Swanson, & Cowan, 1976) and the results obtained by both are very similar. Ascending projections from the VMH can be traced rostrally through the anterior hypothalamus, the periventricular region, the medial hypothalamus, and the medial forebrain bundle (MFB) to the preoptic area, the bed nucleus of the stria terminalis, the anterior amygdaloid area, and the lateral septal nucleus (Krieger et al., 1979; Saper et al., 1976). Saper et al. (1976) reports that efferents reach the ipsilateral amygdala by two distinct pathways; some axons pass through the bed nucleus of the stria terminalis whereas other fibers follow a ventral amygdalopetal route from the lateral hypothalamus and ventral supraoptic commissure. Both sets of fibers terminate in the dorsal portion of the medial amygdaloid nucleus and the capsule of the central nucleus.

Krieger et al. (1979) describe lateral VMH projections which enter the supraoptic commissures and zona incerta and it is suggested that these projections are responsible for labelled fibers in the cerebral peduncle, amygdala, thalamus, and the reticular formation. Medially descending

fibers traverse the posterior hypothalamus and then run dorsally to terminate in the mesencephalic and pontine central grey (Krieger et al., 1979). Saper et al. (1976) suggest that there are three main routes for descending VMH efferents, one through the periventricular system, one through the medial hypothalamus and medial forebrain bundle, and another via the supraoptic commissure. The labelled fibers in the periventricular region project mainly to the posterior hypothalamic area and the central grey, and the VMH projections traversing the medial hypothalamus and MFB terminate in the capsule of the mammillary complex, the supramammillary nucleus and the ventral tegmental area (Saper et al., 1976). Finally, the projections and terminal fields of the fibers coursing through the supraoptic commissure are similar to those described above by Krieger et al. (1979); there are projections to the amygdala, (possibly) the cerebral peduncle, the zona incerta, the central tegmentum, and the peripeduncular nucleus.

Luiten et al. (1987) found that two efferent descending fiber bundles could be identified after the injection of an anterograde tracer into the VMH. The major efferent pathway coursed in a periventricular position and connected directly with the mesencephalic continuation of the of this region, which is the central grey (Luiten et al., 1987). According to these authors, both autoradiographic and *phaseolus vulgaris* leuco-agglutinin (PHA-L) tracing experiments show

that the VMH cells proper do not project much beyond the level of the mesencephalic central grey; therefore, they conclude that this area is the major target of descending VMH projections. Some of these fibers fan out and extend into the dorsal mesencephalic tegmentum. With respect to the other, less dominant, fiber bundle, the projections leave the VMH and run caudally in the floor of the hypothalamus ventral to the medial forebrain bundle. They terminate in the peripeduncular nucleus and, like the fibers from the other pathway, the mesencephalic tegmentum (Luiten et al., 1987). There were also a few VMH connections to the locus coeruleus and the medullary reticular formation (Luiten et al., 1987). The authors emphasize that only a limited amount of labelling was seen at lower brainstem levels with anterograde techniques. However, a very dense retrograde labelling of cell bodies occurred in the VMH when horseradish peroxidase was injected into the central grey; this suggests that the VMH is an important supplier of afferent input to this area (Luiten et al., 1987; Ter Horst, Luiten, & Kuipers, 1984). Ter Horst et al. (1984) go on to suggest that the VMH connects indirectly with parasympathetic motor nuclei via the mesencephalic periaqueductal grey and the parvocellular nucleus of the reticular formation. Swanson and Kuypers (1980), using fluorescent retrograde tracers, found evidence of a direct projection from the VMH to the solitary nucleus and spinal

cord of the rat.

These studies, when considered together, give a reasonably consistent picture of the efferent output of the VMH. It is also apparent that there is a considerable overlap in efferent and afferent VMH projections.

#### **A Closer Look at Intrahypothalamic Circuitry**

The intrahypothalamic connections of the VMH, especially with respect to the LH, have received a great deal of attention ever since Stellar (1954) first proposed the dual center hypothesis. However, anatomical work in this area has been hampered by the fact that a good neuroanatomical tracing technique for examining short distance connections did not exist until the PHA-L tracing method was developed by Gerfen and Sawchenko (1984). Injections of PHA-L into the core of the VMH have revealed the efferent pathways of this nucleus, and injections of the tracer into other hypothalamic areas show the afferent input to the VMH. Specifically, this technique has demonstrated that there are some VMH projections to the ventromedial parts of the LH (in particular the anterior aspects of the medial forebrain bundle); however this output is minor compared to VMH efferent projections to the dorsomedial nucleus and the periventricular zone (Luiten et al., 1987). Another target for VMH output is the peripheral portion of the anterior hypothalamic nucleus. The connections between

both the dorsomedial nucleus and the anterior hypothalamus are reciprocal, with the latter being an especially rich source of intrahypothalamic input to the VMH (Luiten et al., 1987). There are some VMH projections to the premammillary area as well, the main target being the dorsal premammillary nucleus. Injection of tracer into the paraventricular nucleus has revealed projections to the medial portion of the VMH; these connections do not appear to be reciprocal (Luiten et al., 1987). Finally, The VMH has numerous efferent connections within the nucleus itself. The results of the PHA-L studies, taken together, suggest that the VMH is not an important contributor of intrahypothalamic connections because its output is largely restricted to the medial aspects of the dorsomedial nucleus (DMN) and the periventricular column (Luiten et al., 1987). Based on this observation and the efferent connections of the VMH, Luiten et al. (1987) proposed that VMH circuitry is more involved in mediating changes in autonomic output than integrating input within the hypothalamus. It should be emphasized that the present review details only direct efferent and afferent connections of the VMH. Luiten and Room (1980) point out that the absence of direct interconnections between two hypothalamic areas does not eliminate the possibility that they are functionally coupled - information transfer within the hypothalamus undoubtedly occurs via indirect as well as direct connections. The most obvious case in point is the

circuitry involving the LH, VMH, and dorsomedial nucleus. There is a relative lack of direct interconnections between the LH and VMH but the dorsomedial nucleus receives input from, and sends numerous efferents to, both of these nuclei.

The principal afferent and efferent connections of the VMH are summarized in Table 1. The intrahypothalamic connections are also included in these categories. Generally speaking, the projections are listed according to their location along the anteroposterior axis of the rat brain.

#### **Lesion Studies - An Overview**

Much of what we know about the VMH has come from lesion studies. Early on, a series of papers by Hetherington and Ranson (1939, 1940a, 1940b) discussed the effects of bilateral electrolytic lesions of the ventral hypothalamic area, which eliminated the ventral medial nucleus and resulted in obesity in laboratory rats. Furthermore, damage to the pituitary was not necessary for the increase in adiposity (Brobeck, 1946; Hetherington, 1941, 1943; Hetherington & Ranson, 1939, 1940a, 1940b, 1942a, 1942b). In fact, Hetherington (1943) showed that obesity did not develop in hypophysectomized animals if hypothalamic tissue was spared, but these same rats could be made obese by VMH lesions. This particular result put an end to a long

TABLE 1

## Summary of Major VMH Connections

Afferent	Efferent
lateral septal nucleus	preoptic area
bed nucleus of the stria terminalis	bed nucleus of the stria terminalis
medial nucleus of the amygdala	anterior amygdaloid area
central nucleus of the amygdala	lateral septal nucleus
basomedial nucleus of the amygdala	retrochiasmatic area
amygdalo-hippocampal area	medial nucleus of the amygdala
ventral subiculum	capsule of the central nucleus of the amygdala
anterior hypothalamus	anterior hypothalamus
dorsomedial nucleus	dorsomedial nucleus
paraventricular nucleus	periventricular column
periaqueductal grey	premamillary nucleus
peripeduncular nucleus	capsule of mammillary complex
raphe	supramamillary nucleus
parabrachial nucleus	periaqueductal grey
locus coeruleus	peripeduncular nucleus
dorsolateral tegmental area	mesencephalic tegmentum
nucleus incertus	locus coeruleus
nucleus solitarius	nucleus solitarius
lateral reticular nucleus	parvocellular nucleus of the medullary reticular formation
area adjacent to the medial longitudinal fasciculus	upper thoracic spinal cord

Note that intrahypothalamic connections are included in the above categories.

standing debate as to whether hypothalamic damage, or hypopituitarism, was responsible for the development of the obesity (Brobeck, 1946). In addition, hyperphagia and a decrease in locomotor activity were found to be associated with hypothalamic obesity (Brobeck, 1946; Brobeck, Tepperman, & Long, 1943; Hetherington, 1941; Hetherington & Ranson, 1942c; Tepperman, Brobeck, & Long, 1941). These findings, taken in their entirety, served as a starting point for much of the work on the neurobiology of feeding and the etiology of obesity.

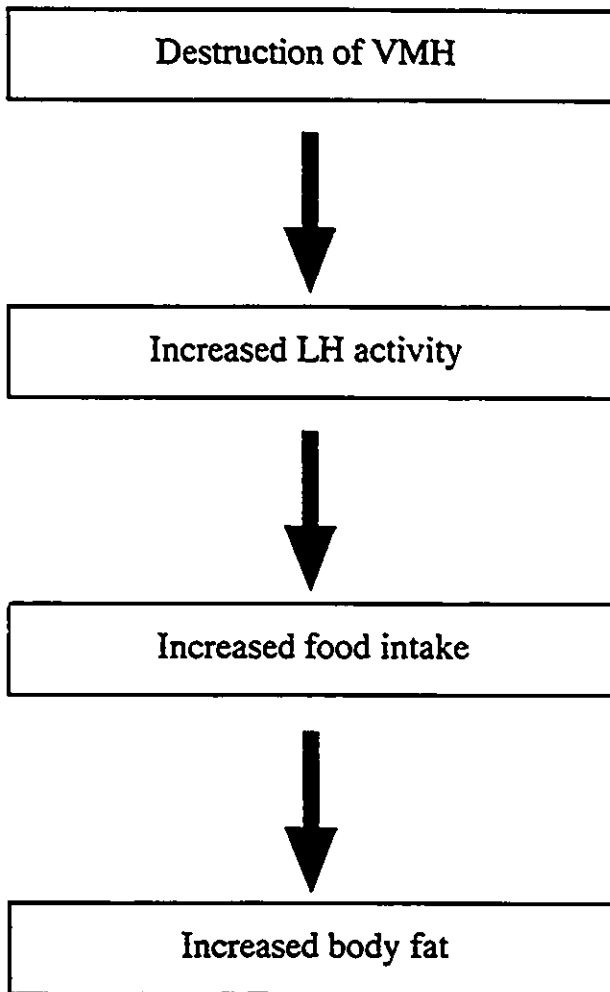
Both the hypophysectomy studies and the hyperphagia observed in VMH damaged animals set the stage for a behavioural interpretation of the process mediating hypothalamic obesity (Powley et al., 1980). Brobeck and his colleagues published a seminal paper in 1943 stating that this hyperphagia was the primary cause of VMH obesity and proposed that metabolic and hormonal changes were secondary to the loss of control over food intake. This notion dominated the literature for many years despite reports that showed enhanced lipogenesis in VMH lesioned animals in the absence of an increase in food intake (Hetherington, 1941; Hetherington & Ranson, 1942c).

Based on this literature, and Anand and Brobeck's (1951) findings concerning lesions of the lateral hypothalamus (LH), Stellar (1954) proposed the "dual center" hypothesis. He suggested that the VMH and the LH were

satiety and feeding centers respectively. It was thought that, in the intact animal, the VMH expressed satiety by directly inhibiting the LH feeding area (Powley, 1977; Stellar, 1954). Destruction of the VMH resulted in a removal of the inhibition and an increase in LH activity. This, in turn, led to an increase in food intake and fat deposition (see Figure 1). Although elegant in its simplicity, several findings have shown that this model may be inadequate. Most significant is the fact that an increased adiposity is observed in VMH lesioned animals even when they are not hyperphagic (Cox & Powley, 1981a; Goldman, Bernardis, & Frohman, 1974; Han, 1967, 1968a, 1968b; Han et al., 1965; Han & Lui, 1966; Han & Frohman, 1970; Brooks & Lambert, 1946; Hetherington, 1941; Hetherington & Ranson, 1942c; Rabin, 1974). In addition, there is evidence that suggests that the hyperphagia is linked to the destruction of fiber tracts running longitudinally between the paraventricular nucleus and brain stem regions, rather than due to the elimination of the VMH per se (Gold, Jones, Sawchenko, & Kapatos, 1977; Kapatos & Gold, 1973). It was proposed that the increased adiposity of lesioned rats, in the absence of hyperphagia, was due to the animals' hypoactivity (Brooks & Lambert, 1946; Hetherington, 1941; Hetherington & Ranson, 1942c). However, later work showed increased fat deposition in VMH damaged rats relative to controls, even when animals were given equal amounts of

Figures 1-3. Figures 1, 2, and 3 illustrate the important events described by the dual center, cephalic phase, and autonomic hypotheses respectively. All three theories attempt to explain the increase in body fat that is observed after VMH lesions. For more detail, refer to Bray (1984).

## Dual Center Model



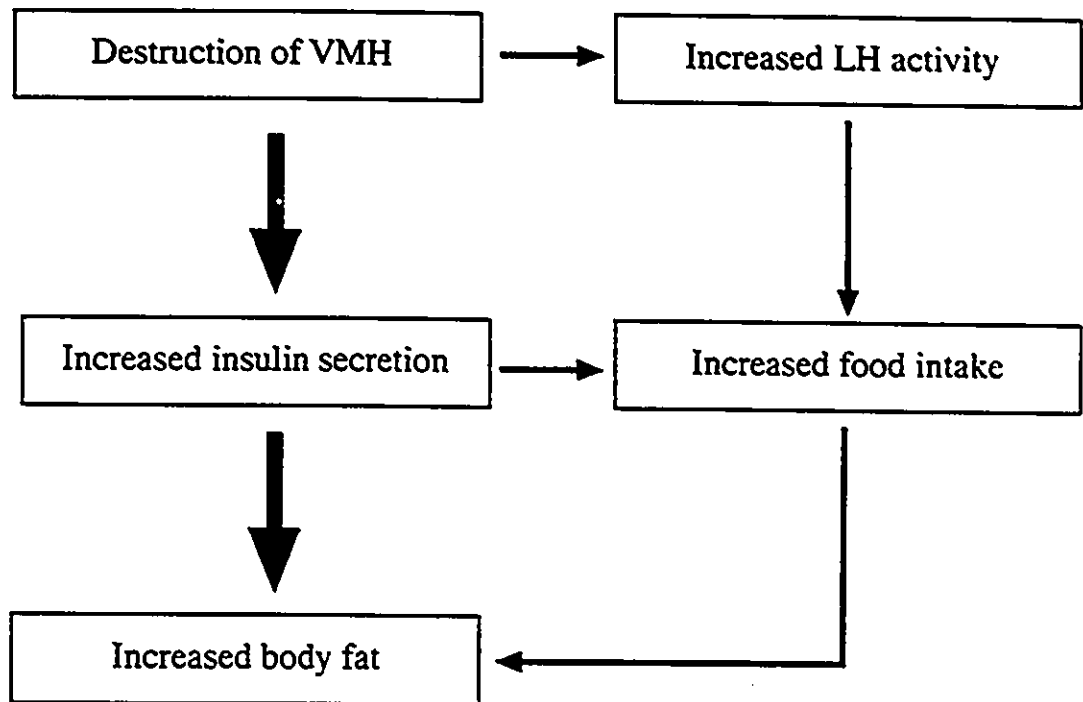
**The dual center model suggests that an increase in body fat is the direct result of an increase in food intake.**

food, and their activity was limited by keeping them in restraining cages (Han, 1968b). Finally, Gold (1973) found that lesions confined to the VMH did not lead to substantial weight gain in rats and suggested that the VMH had been singled out merely because it is the most prominent structure in the basomedial hypothalamus.

At an anatomical level, some support was found for the dual center hypothesis (Arees & Mayer, 1967; Eager, Chi, & Wolf, 1971) but later studies showed that fibers that appear to underlie the expression of the VMH syndrome run in a rostro-caudal direction rather than directly between the VMH and LH areas (Gold et al., 1977; Kapatos & Gold, 1973; Sclafani & Maul, 1974).

Powley (1977) tried to address some of these inconsistencies in the cephalic phase hypothesis (see Figure 2). This model provided a framework within which both the behavioural aspects (e.g. finickiness) and the metabolic changes (e.g. hyperinsulinemia) associated with VMH obesity could be explained - even when food intake is strictly controlled. It was proposed that a mechanism that links sensory events to metabolic processes was responsible for the distinguishing features of the syndrome (Powley, 1977). The primary consequence of VMH lesions was therefore thought to be an exaggeration of the efferent limb of the cephalic reflexes of digestion (Powley, 1977). In contrast to the dual center model, which views hyperphagia as the

## Cephalic Phase Hypothesis



**According to the cephalic phase model, the increase in body fat is the result of enhanced cephalic reflexes; in particular, an increase in insulin secretion.**

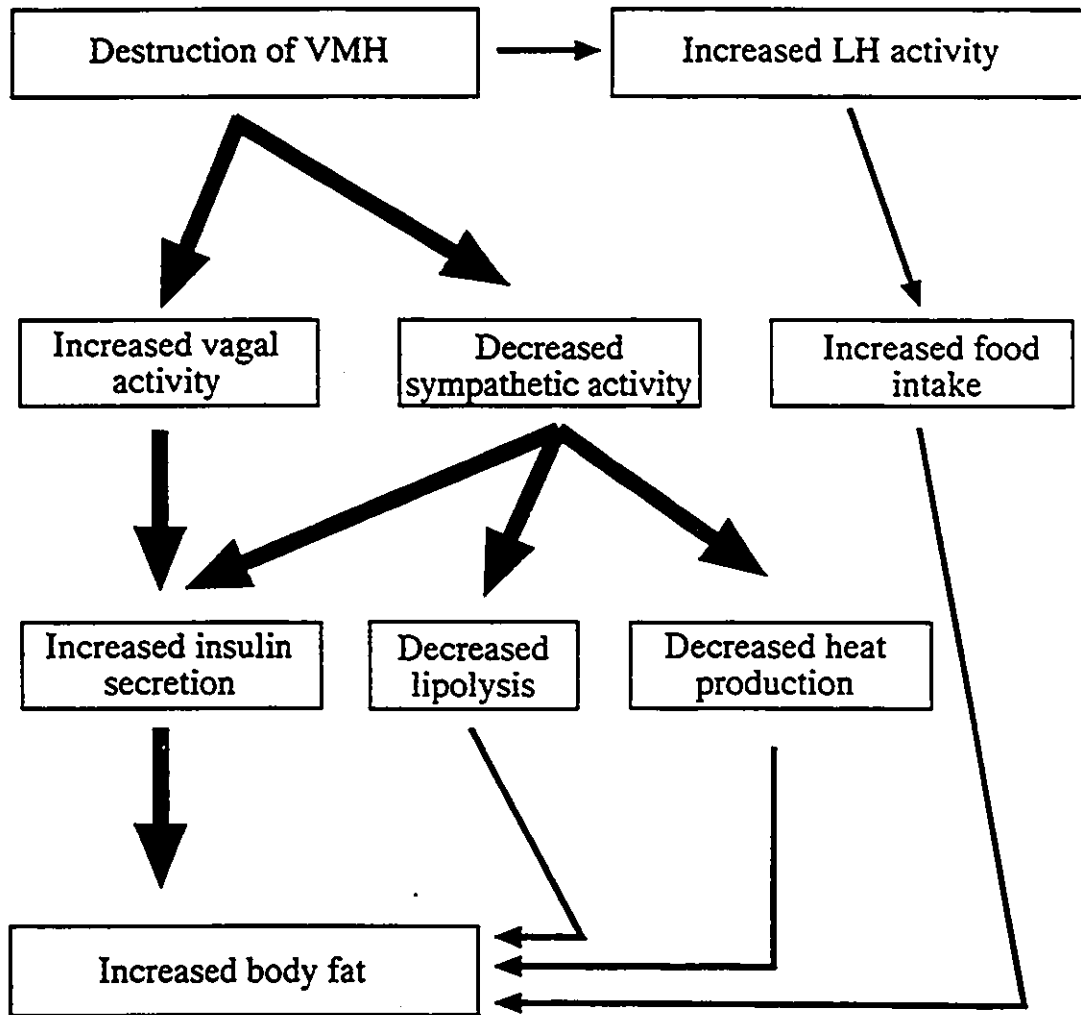
primary cause of VMH obesity, the cephalic phase hypothesis maintains that the increase in food intake (and obesity) is secondary to the changes in energy metabolism produced by a lesion-induced augmentation of the cephalic reflexes (Powley, 1977). In other words, the VMH lesioned rat will have exaggerated salivary, gastrointestinal, pancreatic, and hepatic adjustments compared to the intact animal; the net effect of these adjustments is a shift in energy metabolism towards anabolism and fat storage. An increase in insulin secretion is the key element in this metabolic shift since it promotes lipogenesis, glycogenesis, and hypoglycemia (Powley, 1977). Hence, Powley suggested that the rat becomes hyperphagic and grows obese in response to lesion-induced hyperinsulinemia.

Powley (1977) acknowledged that various autonomic and endocrine responses, in addition to cephalic reflexes, may be affected by VMH lesions. Consistent with this notion is the finding that subdiaphragmatic vagotomy attenuates, or even reverses, the effects of VMH lesions (Berthoud & Jeanrenaud, 1979; Inoue & Bray, 1977; King, Carpenter, Stamoutsos, Frohman, & Grossman, 1978; Powley & Opsahl, 1974). Cox and Powley (1981b) found that prior vagotomy prevents the development of VMH obesity in pair-fed rats, supporting the idea that the vagus nerve plays a pivotal role in the emergence of lesion-induced hyperinsulinemia. Work by Inoue and colleagues (1977, 1978) highlights the

importance of neural outputs from the VMH to the  $\beta$ -cells of the pancreas in the development of hyperphagia, hyperinsulinemia, and obesity. They found that animals made diabetic with streptozocin, and which subsequently received fetal pancreatic tissue transplants beneath the renal capsule prior to VMH lesioning, had an attenuated obesity, hyperinsulinemia, and food intake relative to controls. Hence, all of these studies suggest that VMH lesions result in increased vagal activity which then leads to an increase in insulin, hyperphagia, and obesity.

The autonomic hypothesis (Bray & York, 1979) represents an extension of the cephalic phase/vagal model (see Figure 3). It proposes that there is an imbalance between the parasympathetic and sympathetic nervous systems; the former is hyperactive whereas sympathetic activity is reduced. Within this context, VMH obesity is thought to be the result of an increased stimulation of pancreatic insulin secretion mediated by increased parasympathetic (excitatory) and decreased sympathetic (inhibitory) nervous activity (Inoue, Campfield, & Bray, 1977; Inoue & Bray, 1980; Smith & Campfield, 1986; Tokunaga et al., 1986). The first indications of a depressed sympathetic function came from work showing that VMH-lesioned rats have a lower concentration of circulating glucagon and smaller salivary glands relative to control animals (Chikamori et al., 1980; Inoue, Campfield, & Bray, 1977). It was also found that the

## Autonomic Hypothesis



According to the above model, changes in the autonomic nervous system are viewed as the principal factors in the development of obesity.

mobilization of free fatty acids and the release of dopamine  $\beta$ -hydroxylase (an enzyme reported to provide an index of sympathetic activity) are both reduced in VMH damaged rats when the animals are exposed to different stressors (Bray, 1984; Inoue & Bray, 1980; Nishizawa & Bray, 1978). In addition, Vander Tuig and colleagues (1982) saw a lower rate of norepinephrine turnover in heart, liver, brown fat, and pancreas in VMH-lesioned weanling rats than in control animals. However, the role of the sympathetic nervous system in the development of hypothalamic obesity is not without controversy. Yoshida and Bray (1984) were not able to replicate the finding of Vander Tuig et al. (1982). In fact, they observed that the rate of norepinephrine turnover was significantly greater in the heart and the brown adipose tissue of VMH-lesioned rats. They also found the functioning of the pancreas to be unaltered by VMH-lesions (Yoshida & Bray, 1984). Furthermore, fasting, which slowed the rate of norepinephrine turnover in control rats, had no effect in VMH-lesioned animals. These results, while at variance with the autonomic hypothesis, are consistent with other work; Young and Landsberg (1980) found that cardiac norepinephrine turnover rate was not altered by fasting in mice with gold thioglucose lesions and Young, Saville, Rothwell, Stock, and Landsberg (1982) showed cold exposure significantly increased the norepinephrine turnover rate in both lesioned and control animals. Hence, one prediction of

the autonomic hypothesis, that a reduced sympathetic activity should result in a decrease in norepinephrine turnover in peripheral tissues, is not upheld. One explanation that has been proposed for this outcome is that the hyperinsulinemia observed in the VMH syndrome (which is mainly due to an increased vagal stimulation of the pancreatic  $\beta$ -cells) may augment norepinephrine turnover but also decrease norepinephrine responses - one of which is lipolysis (Yoshida & Bray, 1984). Bray (1984) adds a slightly different twist to this interpretation by proposing that while the hyperphagia and the hyperinsulinemia associated with VMH lesions may be the stimuli that increase the rate of norepinephrine turnover, it is the increased levels of insulin that are responsible for the decreased lipolysis (rather than the direct effect of altered sympathetic tone). However, Duggan & Booth (1986) have found that there is a sympathetic component to the hyperinsulinemia following VMH lesions. This reinforces the notion that the symptoms of hypothalamic obesity are the result of altered activity levels in both the parasympathetic and sympathetic branches of the autonomic nervous system.

The finding that lateral hypothalamic lesions result in an increased sympathetic tone, as indicated by an increased norepinephrine turnover in heart and BAT (Yoshida, Kemnitz, & Bray, 1983), has resulted in the "modified autonomic

hypothesis". According to this model, the activity of the sympathetic nervous system is modulated by inputs from the LH, insulin and food intake. The VMH, on the other hand, modulates vagal efferent tone and has an inhibitory influence on the LH. It, in turn, receives input from the suprachiasmatic nucleus. This would explain the loss of diurnal oscillations in food intake following VMH lesions (Bray, 1984; Powley, Opsahl, Cox, & Weingarten, 1980). Hence, the two hypothalamic centers (the VMH and the LH) appear to have key roles in the modulation of the autonomic nervous system which in turn regulates energy balance and metabolism.

Although there are still unanswered questions regarding the exact etiology of the VMH syndrome, work in this area has proven invaluable in broadening our knowledge of metabolism and food intake. Perhaps most importantly, these studies have provided a foundation for the development and evolution of a concise model of metabolism, obesity, and food intake (such as the modified autonomic hypothesis). Another research strategy has been to examine the effects of VMH stimulation; the results of these studies have complimented and expanded the knowledge base generated by the lesion work.

## VMH Stimulation

### Glucose Homeostasis

Generally speaking, both electrical and chemical stimulation of the VMH produces effects which are opposite to those seen in hypothalamic obesity. Frohman and Bernardis (1971) found that VMH stimulation results in an increase in plasma glucose and plasma glucagon levels in anaesthetized rats. Plasma insulin remains at basal levels, or decreases during the stimulation, despite an increase in blood glucose (Frohman & Bernardis, 1971; Shimazu, Matsushita, & Ishikawa, 1978). Furthermore, Frohman & Bernardis (1971) showed that plasma insulin levels increase, presumably in response to elevated blood glucose levels, when VMH stimulation is terminated. Their results also revealed that the sympathetic inhibition of insulin secretion is primarily mediated by the adrenal gland since, after adrenalectomy, plasma insulin levels rose and fell in parallel with plasma glucose levels (Frohman & Bernardis, 1971). Presumably epinephrine is responsible for the decreased insulin secretion since it is known to inhibit the release of insulin through an  $\alpha$ -adrenergic mechanism (Frohman, 1980; Porte, 1969). It was also suggested that the direct neural inhibition of pancreatic  $\beta$ -cells is unimportant in the control of insulin secretion, and indeed, the rapid increase in plasma insulin observed in response to

rising blood glucose levels in VMH-stimulated adrenalectomized animals supports this notion (Frohman & Bernardis, 1971). However, there is evidence of species variability; sympathetic nerve stimulation has been shown to have a direct inhibitory effect on insulin secretion in dogs (Frohman, 1980; Miller, 1975). In humans, direct neural inhibition of insulin release is more important than inhibition via adrenal catecholamines (Frohman, 1980). Also, it is known that the pancreatic islets are richly innervated with both sympathetic and parasympathetic fibers in most vertebrate species (Frohman, 1980; Woods & Porte, 1974). What is consistent across species is the finding that the inhibition of insulin secretion is associated with the  $\alpha$ -adrenergic receptor of the pancreatic  $\beta$  cell; phentolamine (an  $\alpha$ -receptor blocking agent) antagonizes the suppressive effects whether they are adrenally mediated, as in the rat, or neurally mediated, as in the dog. (Frohman, 1980; Frohman et al., 1974; Baum & Porte, 1971).

Frohman and Bernardis (1971) examined the possibility that VMH stimulation alters the rate of peripheral glucose uptake by measuring the disappearance rate of  $^{14}\text{C}$ -glucose from plasma during the development of hyperglycemia. Results showed that the rate of glucose disappearance was similar in VMH stimulated and control groups, suggesting that the hyperglycemia was due to hepatic glucose output, rather than to net changes in peripheral glucose uptake

(Frohman & Bernardis, 1971). These findings were confirmed by Shimazu and Ishikawa (1981) who looked at the effects of electrical and chemical VMH stimulation on glucagon and insulin secretion in conscious, free-moving rabbits. Electrical stimulation of the VMH elicited glucagon secretion from the pancreas and, during the stimulation, suppressed the release of insulin (Shimazu & Ishikawa, 1981). Administration of epinephrine, via a cannula implanted in the VMH, resulted in rapid increases in plasma levels of glucagon and glucose followed by an increase in insulin; this is a similar profile to that observed after electrical stimulation of the VMH (Shimazu & Ishikawa, 1981). Chemical stimulation using the same dose of norepinephrine led to a much smaller increase in plasma levels of glucose, glucagon, and insulin, whereas stimulation with acetylcholine elicited a selective release of glucagon which was then followed by a gradual rise in blood glucose (Shimazu & Ishikawa, 1981). The effects of acetylcholine appear to be mediated by nicotinic receptors since intrahypothalamic injection of hexamethonium, but not atropine, attenuated the glucagon response and hyperglycemia (Shimazu & Ishikawa, 1981). Jong, Strubbe, and Steffens (1977) found that noradrenergic stimulation of the VMH resulted in elevated plasma glucagon and insulin levels, and increased blood glucose levels in rats. They also concluded that  $\alpha$ -receptors are primarily responsible for the increase

in both insulin and glucagon secretion (norepinephrine acts primarily at the  $\alpha$ -receptor) since chemical stimulation of the VMH by injection of isoproterenol, a  $\beta$ -sympathomimetic agent, did not result in a significant increase in insulin and glucagon release (Jong et al., 1977).

It is clear that stimulation of the VMH results in glycogenolysis in the liver, as indicated by a rapid increase in the level of plasma glucose and decrease in liver glycogen content (Frohman & Bernardis, 1971; Shimazu, 1981; Shimazu, Fukuda, & Ban, 1966; Shimazu & Ishikawa, 1981). Consistent with these findings is the observation that glycogen phosphorylase-a and glucose-6-phosphatase both show a rapid increase in activity after electrical stimulation of the VMH (Fohman, 1980; Shimazu et al., 1966; Shimazu, 1981; Shimazu, Matsushita, & Ishikawa, 1978). Glycogen phosphorylase and glucose-6-phosphatase are both hepatic enzymes; the former exists in two interconvertible forms, the physiologically active form, phosphorylase-a, and the inactive form, phosphorylase-b. Glycogen phosphorylase-a reflects the rate of glycogen breakdown in the liver, and glucose-6-phosphatase converts glucose-6-phosphate to glucose in preparation for release into the blood.

Chemical stimulation of the VMH has revealed that norepinephrine also elicits an increase in the conversion of the inactive form of liver phosphorylase to the active form (Matsushita & Shimazu, 1980; Shimazu, 1981). In addition,

it has been shown that the response profile of liver phosphorylase is similar for both electrical and chemical stimulation of the VMH (Matsushita & Shimazu, 1980). Stimulation with dopamine was found to decrease phosphorylase-a activity by about 20%; other neurotransmitters (serotonin, acetylcholine, and  $\gamma$ -aminobutyric acid) had no effect on phosphorylase-a activity (Matsushita & Shimazu, 1980). Intracerebral injections into the VMH with propranolol (a  $\beta$ -receptor blocking agent), but not phentolamine (an  $\alpha$ -receptor blocking agent), was found to suppress the activation of liver phosphorylase after noradrenergic stimulation of the VMH; this suggests that norepinephrine sensitive neurons in the VMH are involved in the regulation of hepatic phosphorylase activity, and that the effects of chemical stimulation are mediated by  $\beta$ -adrenergic receptors in the hypothalamus (Matsushita & Shimazu, 1980; Shimazu, 1981). In addition, it was found that stimulation-induced activation of liver phosphorylase is prevented by a prior interperitoneal injection of the ganglionic blocker hexamethonium (Matsushita & Shimazu, 1980; Shimazu, 1981). This latter finding suggests that the effects of noradrenergic stimulation of the VMH are mediated by peripheral sympathetic activity (Matsushita & Shimazu, 1980; Shimazu, 1981). The fact that studies have shown that splanchnic nerves exert direct control over liver

phosphorylase activity supports this claim (Shimazu & Amakawa, 1968a, 1968b, 1975).

Ventromedial hypothalamic stimulation has also been found to alter the activity of hepatic gluconeogenic and glycolytic enzymes; specifically, it increases the activity of the gluconeogenic enzyme, phosphoenolpyruvate carboxylase, and suppresses the activity of the glycolytic enzyme, pyruvate kinase (Shimazu & Ogasawara, 1975; Shimazu, 1981). The net result of these changes in enzymatic activity is an increase in liver glucose production. This outcome complements stimulation-induced hepatic glycogenolysis (discussed earlier) and contributes to the hyperglycemic response (Shimazu, 1981; Shimazu & Ogasawara, 1975). However, unlike the glycogenolytic responses to VMH stimulation, which are very rapid (Frohman & Bernardis, 1971; Shimazu et al., 1966; Shimazu et al., 1978) the gluconeogenic responses are much slower (Shimazu, 1981; Shimazu & Ogasawara, 1975). It is suggested that the former are central to preparing the animal for a fight or flight response, whereas gluconeogenic responses are less involved in activating the organism in an emergency situation (Shimazu, 1981; Shimazu & Ogasawara, 1975).

### **Glucose Transport**

Obviously, if circumstances arise that jeopardize an animal's existence, it is crucial that circulating blood

glucose be made available to peripheral tissues. The rate-limiting step in glucose utilization, especially in insulin sensitive tissues such as muscle, is the transport of glucose across the cell membrane. In certain peripheral tissues, electrical and chemical (L-glutamate) stimulation of the VMH has been found to significantly increase insulin-independent glucose uptake as measured by the ( $^3\text{H}$ )2-deoxyglucose (2-DG) method (Shimazu, Sudo, Minokoshi, & Takahashi, 1991; Sudo, Minokoshi, & Shimazu, 1991; Takahashi et al., 1992). Specifically, both heart and skeletal muscles and brown adipose tissue (BAT) show a stimulation-induced increase in ( $^3\text{H}$ )2-DG uptake in anaesthetized rats (Shimazu et al., 1991; Sudo et al., 1991; Takahashi et al., 1992). On the other hand, VMH stimulation was found to have no effect on the rate of ( $^3\text{H}$ )2-DG uptake in white adipose tissue or the diaphragm (Shimazu et al., 1991; Sudo et al., 1991). Local sympathetic denervation eliminated the increase in ( $^3\text{H}$ )2-DG uptake in BAT suggesting that this effect is mediated by sympathetic nerve activity rather than an insulin dependent mechanism of glucose transport (Shimazu et al., 1991; Sudo et al., 1991; Takahashi et al., 1992). This is consistent with previous work that has shown that plasma insulin levels remain unchanged or are suppressed by VMH stimulation (Frohman & Bernardis, 1971; Shimazu & Ishikawa, 1981). Furthermore, Sudo et al. (1991) confirmed that neither electrical or chemical stimulation of the VMH

elicited an increase in plasma insulin levels; therefore, insulin could not be responsible for the increased rate of glucose transport. They suggest that elevated tissue glucose uptake may be related to changes in blood flow since VMH stimulation has been reported to increase the regional blood flow of BAT and skeletal muscle (Iwai, Hell, & Shimazu, 1987).

Glucose transporters are estimated by the D-glucose-inhibitable ( $^3\text{H}$ )cytochalasin B binding to microsomal or plasma membranes (Cushman & Wardzala, 1980). Scatchard analysis of the D-glucose inhibitable binding of ( $^3\text{H}$ )cytochalasin B shows that both the number and the affinity of glucose transporters remains unchanged with VMH stimulation; this is true for both plasma and microsomal membrane fractions isolated from heart or BAT (Shimazu et al., 1991; Takahashi et al., 1992). Insulin treatment, on the other hand, increases the number of glucose transporters in plasma membranes and decreases those in microsomal membranes; it had no effect on the affinity of glucose transporters (Shimazu et al., 1991; Takahashi et al., 1992). This is consistent with the notion that insulin increases glucose transport by augmenting the number of glucose transporters at the plasma membrane via recruitment from the intracellular pool (Cushman & Wardzala, 1980; Suzuki & Kono, 1980).

Since neither the number of glucose transporters nor their affinity are altered by VMH stimulation, it is likely that an increase in the intrinsic activity and/or turnover rate of membrane transporters mediates the increased glucose transport (Shimazu et al., 1991). Takahashi et al. (1992) found support for this line of reasoning; they estimated the apparent functional activity of glucose transporters (D-glucose transport activity per glucose transporter) by calculating the ratio of specific D-glucose transport to glucose transporter number. Specific D-glucose transport was determined by subtracting nonspecific glucose transport, measured by L-(<sup>14</sup>C)glucose, from D-(<sup>14</sup>C)glucose. The measurements were taken under equilibrium exchange conditions using plasma membrane vesicles prepared from heart and BAT (Takahashi et al., 1992). Glucose transporters were estimated as previously described, using D-glucose-inhibitable (<sup>3</sup>H)cytochalasin B binding (Cushman & Wardzala, 1980; Takahashi et al., 1992). Results showed that specific D-glucose transport was significantly increased in both types of plasma membrane vesicles (BAT and heart) in VMH stimulated rats compared to control animals (Takahashi et al., 1992). However, no significant difference was observed between control and insulin treated rats in specific D-glucose transport for either membrane fraction (Takahashi et al., 1992). This provides further support for the claim that the mechanism by which VMH

stimulation increases glucose uptake is different from that of insulin.

Recently, Minokoshi, Okano, and Shimazu (1994) showed that electrical stimulation of the VMH increases glucose uptake in skeletal muscles, BAT, and heart, even when muscle contraction is blocked by an iv injection of the muscle relaxant pancuronium bromide. This suggests that VMH stimulation increases the rate constant of glucose uptake independent of muscle contraction (Minokoshi et al., 1994). They believe that this outcome is unlikely to be simply the result of stimulation induced hyperglycemia because an increase in the rate constant for glucose uptake was not observed after electrical stimulation of the posterior or paraventricular hypothalamic nucleus; this despite the fact that stimulation of these same areas resulted in an increase in blood glucose. It is suggested that the effect of VMH stimulation (accelerated glucose uptake in muscle, BAT, and heart) may be mediated by sympathetic nerves because it is abolished by pretreatment with the postganglionic sympathetic blocker guanethidine, but not by bilateral adrenalectomy (Minokoshi et al., 1994). This is consistent with the findings concerning the effects of local sympathetic denervation (Shimazu et al., 1991; Sudo et al., 1991; Takahashi et al., 1992). Therefore, it seems that the VMH plays an important role in regulating glucose utilization in skeletal muscles, and sympathetic nerves are

the intermediary through which this is accomplished.

The work concerning the effects of VMH stimulation on glucose transport, taken together, shows that the mechanism by which VMH stimulation increases glucose uptake in peripheral tissues is different from the one triggered by insulin administration (Minokoshi et al., 1994; Shimazu et al., 1991; Sudo et al., 1991; Takahashi et al., 1992). It has been suggested that both VMH stimulation and exercise promote glucose uptake via a similar mechanism (Shimazu et al., 1991; Takahashi et al., 1992), and in fact, it has been found that exercise increases the intrinsic activity of rat sarcolemmal glucose transporters (Sternlicht, Barnard, & Grimditch, 1989). In addition, work with plasma membrane vesicles isolated from rat skeletal muscles has shown an increase in both the number and the intrinsic activity of glucose membrane transporters following exercise or muscle contraction (Goodyear, King, Hirshman, Thompson, Horton, & Horton, 1990; King, Hirshman, Horton, & Horton, 1989). Therefore, there is an accumulating body of evidence which supports the idea that the non-insulin dependent uptake of glucose, elicited by both VMH stimulation and exercise, is mediated by a similar mechanism. Finally, perhaps one of the most interesting facts which emerges from this research is that the VMH is involved in the modulation of glucose utilization as well as hepatic glucose production (Shimazu, 1991).

### Lipolysis and Lipogenesis

Both VMH lesion and stimulation studies indicate that the VMH, and the sympathetic descending pathways to the pancreas, liver, and adipose tissues, control lipid metabolism (Le Magnen, 1992). Kumon, Takahashi, Hara and Shimazu (1976) found that plasma glycerol levels were increased after a single (one minute) stimulation of the VMH in conscious rabbits. The maximum value was reached 20 minutes after termination of the stimulation. Surprisingly, since the hydrolysis of triglycerides yields glycerol and free fatty acids, they saw no increase in plasma free fatty acids (Kumon et al., 1976). It is suggested that this may be due to either an accelerated utilization of free fatty acids, since VMH stimulation in unanaesthetized animals elicits excitement and motor activity, or, to elevated levels of plasma lactate - lactate inhibits the release of free fatty acids from adipose tissue by increasing the rate of reesterification (Kumon et al., 1976; Shimazu, 1981). Consistent with the work examining the effects of VMH stimulation on blood glucose levels (Frohman & Bernardis, 1971; Shimazu & Ishikawa, 1981; Jong et al., 1977), Kumon et al. (1976) saw a stimulation induced increase in plasma glucose. However, plasma glucose levels reached a maximum value ten minutes before maximum plasma glycerol levels were obtained (Kumon et al., 1976). It was concluded, due to this time lag between peak plasma glycerol and glucose

concentrations, that the lipolytic response to VMH stimulation is slower than the glycogenolytic one, and that each outcome is caused by a different mechanism (Kumon et al., 1976). Kumon et al. (1976) also found that the stimulation-induced increases in plasma glycerol were suppressed after treatment with either the ganglionic blocker hexamethonium or the  $\beta$ -adrenergic blocker propranolol; treatment with the  $\alpha$ -adrenergic blocker phentolamine, or the anti-cholinergic drug atropine, had no effect on the elevated plasma glycerol levels. In addition, bilateral adrenalectomy eliminated approximately 80% of the increase in plasma glycerol elicited by VMH stimulation and this effect was not prevented by glucocorticoid replacement (Kumon et al., 1976; Kumon, Takahashi, & Kori-Hara, 1977; Shimazu, 1981). These findings suggest that, in rabbits, the effect of electrical stimulation of the VMH is hormonally mediated by catecholamines released from the adrenal medulla, and that these catecholamines act on the  $\beta$ -receptors of adipose tissue (Kumon et al., 1976). A later study showed the adrenal catecholamine responsible for this effect was epinephrine (Kumon et al., 1977).

Electrical stimulation of the VMH in conscious rats has confirmed the findings observed in rabbits; plasma glycerol was increased but no change was detected in plasma free fatty acid levels (Barkai & Allweis, 1972; Shimazu et al., 1981). However, in anaesthetized rats, both plasma glycerol

and free fatty acid levels were significantly increased (Shimazu et al., 1981). Furthermore, bilateral adrenalectomy did not prevent the lipolytic response to VMH stimulation suggesting a species difference in the mediation of the response (Shimazu, 1981). In agreement with the work done in rabbits, the lipolytic activity was completely blocked by hexamethonium or propranolol, but not with phentolamine. These results imply that (in the rat) sympathetic innervation of adipose tissue through a  $\beta$ -adrenoceptor mechanism is the dominant factor in VMH-induced lipolysis (Niijima, 1989; Shimazu et al., 1981). Chemical stimulation of the VMH also appears to trigger lipolysis since infusion of epinephrine or norepinephrine into the VMH elicits an increase in plasma free fatty acid levels (Niijima, 1986; Steffens et al., 1984).

Surprisingly, electrical stimulation of the VMH has been found to increase lipid synthesis (as measured by the incorporation of tritium from  $^3\text{H}_2\text{O}$  into fatty acids) preferentially in brown adipose tissue; stimulation-induced lipogenesis was not observed in white adipose tissue or the liver (Shimazu & Takahashi, 1980; Shimazu, 1981). It is unlikely that this lipogenesis is mediated by insulin since it has been shown that VMH stimulation inhibits insulin secretion (Frohman & Bernardis, 1971; Shimazu et al., 1978). Furthermore, rats made diabetic by streptozotocin treatment still show a stimulation-induced increase in BAT lipogenesis

(Shimazu & Takahashi, 1980; Shimazu, 1981). It is suggested that the lipogenic effect may be mediated by the sympathetic innervation of BAT, and indeed it has been shown that BAT is richly innervated with sympathetic adrenergic fibers; white adipose tissue, on the other hand, contains fewer adrenergic fibers (Daniel & Derry, 1969; Derry et al., 1969; Shimazu & Takahashi, 1980; Shimazu, 1981).

Perkins, Rothwell, Stock, and Stone (1981) showed that electrical stimulation of the VMH of anaesthetized rats produced a biphasic response in interscapular BAT temperature; an initial decrease was followed by a much larger increase in temperature. No significant changes were observed in skeletal muscle or rectal temperatures (Perkins et al., 1981). Treatment with the  $\alpha$ -antagonist phentolamine inhibited the initial decrease in temperature but did not affect the later rise, whereas intravenous injection of the  $\beta$ -adrenergic blocker propranolol largely eliminated the increase in BAT temperature (Perkins et al., 1981). They propose that VMH activation of BAT is mediated by  $\beta$ -adrenergic receptors, which is consistent with the finding that the increased metabolic rate of animals exhibiting non-shivering or shivering thermogenesis is attenuated by  $\beta$ -antagonists but not affected by  $\alpha$ -antagonists (Perkins et al., 1981). The observation that topical application of a local anaesthetic (tetracaine) to interscapular BAT abolished the stimulation-induced rise in BAT temperature,

whereas an ip injection of norepinephrine elicited the biphasic temperature response, led to the conclusion that central stimulation of BAT thermogenesis is mediated by the sympathetic nerve supply to this tissue (Perkins et al., 1981). More recent studies have verified the importance of the VMH in the control of BAT activity (Himms-Hagen, 1989; Holt, Wheal, & York, 1987, 1988; Iwai, Hell, & Shimazu, 1987; Kelly & Bielajew, 1991; Minokoshi, Saito, & Shimazu, 1986; Rothwell, 1989; Saito, Minokoshi, & Shimazu, 1987, 1989; Sakaguchi & Bray, 1987). Ventromedial hypothalamic stimulation has been shown to raise BAT temperature in conscious and anaesthetized animals, elicit an increase in norepinephrine turnover in BAT, and increase blood flow to BAT (Kelly & Bielajew, 1991; Iwai et al., 1987; Perkins et al., 1981; Rothwell, 1989; Saito et al., 1987; Saito et al., 1988). These findings, plus the fact that the control of BAT thermogenesis is abnormal in most animal models of obesity (including hypothalamic obesity), have led to the suggestion that BAT thermogenesis may be central to the maintenance of leanness, or, when it is compromised, help promote obesity (Himms-Hagen, 1984; Himms-Hagen, 1989). This line of reasoning is supported by studies showing that VMH stimulation is associated with an increase in energy expenditure (Atrens, Sinden, Penicaud, Devos, & Le Magnen, 1985; Pawson, Preston, Haas, & Foster, 1987). Atrens et al. (1985) found that electrical stimulation of the VMH elicited

an increase in energy expenditure, as measured by whole body oxygen consumption, in conscious rats. They conclude that this hypermetabolism may be due to BAT activation or some other form of non-shivering thermogenesis (Atrens et al., 1985). Pawson et al. (1987) also found that rats receiving VMH stimulation showed an increase in metabolic rate. Specifically, they observed a greater than 90% increase in metabolic rate when the VMH or anterior hypothalamus were stimulated; stimulation of other hypothalamic areas produced a lesser response. Furthermore, when the animals were mildly sedated with Xylazine (Rompun), to eliminate the contribution of arousal and body movement to metabolic rate, only the VMH and the posterior portion of the anterior hypothalamus showed a significant increase in metabolic rate (40-60%). These latter studies indicate that the VMH is a key player, not only in the regulation of energy balance, but also in the modulation of energy expenditure. The work of Pawson (1988a) supports this view; he found that chronic VMH-area stimulation resulted in reduced weight gain in normophagic rats and suggests that this may be due to an increase in energy expenditure.

## RATIONALE

Acute stimulation of certain regions of the anterior and ventromedial hypothalamus in the rat results in a greater than 90% increase in metabolic rate as measured by whole body oxygen consumption (Pawson et al., 1987). Moreover, when the effects of arousal and muscle activity are eliminated by an injection of the anaesthetic Rompun, an increase in metabolic rate of 40-60% is still observed (Pawson et al., 1987). This suggests, as discussed earlier, that these regions are important in the regulation of both energy balance and expenditure.

Consistent with the results of the above studies, chronic stimulation of the VMH has been shown to be associated with a reduced weight gain in normophagic rats (Pawson, 1988a). The principal concerns of this thesis are to examine further the effects of chronic VMH stimulation and to clarify the degree to which the decreased weight gain can be attributed solely to stimulation of the VMH. The acute work, specifically that of Pawson et al. (1987), points to the need for an adequate experimental control in chronic studies. They showed that about 50% of their experimental effect (i.e. the increase in metabolic rate due to VMH stimulation) was lost when the animals were sedated with Rompun. This suggests that both locomotor activity and muscle tonus contribute significantly to the stimulation

induced increase in metabolic rate. Pawson (1988a) proposed that the reduced weight gain observed with chronic VMH stimulation is due to an elevation in energy expenditure, and it follows that one aspect of this increase would involve stimulation induced changes in motor activity. In his chronic study the weight gain of rats receiving repeated VMH stimulation was compared to that of a control group that received no stimulation (Pawson, 1988a). It is difficult to determine what percentage of the reduced weight gain can be attributed specifically to VMH stimulation because a stimulated control group was not included. Electrical brain stimulation, in general, may lead to increased arousal and motor activity which in turn influences weight gain. Therefore, in the present work, the determination of a suitable stimulated control was of primary concern. This issue, as well as the resolution of technical and methodological problems, were addressed in the pilot phase of these studies.

### Pilot Phase

The principal objective of the pilot phase was to find a suitable stimulated control against which the VMH stimulated group could be compared. Hence, this work lays the foundation for Experiments 1 and 2 of the thesis by defining the groups to be studied and the methodology to be used.

Figure 4 shows the growth curves for 14 rats implanted with a single electrode aimed at the VMH, the LH, or the region immediately surrounding the VMH (extra-VMH). There were four animals in each of the VMH and LH groups, and six in the extra-VMH group. It is apparent from Figure 4 that the growth curves for the three groups are very similar. In addition, the epididymal fat pad weights (fat pads were removed and weighed after sacrifice) were comparable across the groups (see Table 2). However, on closer examination of Figure 4, it might be argued that there was a slight tendency for rats receiving VMH stimulation to gain weight more slowly than those implanted in other areas because by the end of the stimulation phase (trial 13) the mean body weight of the VMH group was less than either of the control groups - a trend that persisted for more than a week following termination of the stimulation.

Figure 5 shows the growth curves for two groups of rats (n=5 animals per group) with single electrode implants in

**Figure 4.** Growth curves for three groups of rats receiving low levels of electrical brain stimulation. A single electrode was implanted in one of three brain regions; the VMH, the LH, or the area adjacent to the VMH (extra-VMH). There were four animals in each of the VMH and LH groups, and six in the extra-VMH group. Each point represents the mean group weight  $\pm$  S.E.M. The error bars are not shown because of the degree of overlap among groups. The S.E.M. ranges for the VMH, LH, and extra-VMH groups were 1.3-15.8, 8.0-22.9, and 8.6-18.8 respectively, values that were roughly proportional to their associated means. Stimulation trials 2-13 were administered three times a week over four weeks, after which rats were monitored for an additional three weeks. The first trial was interrupted because the rats showed an excessive level of stimulation bound activity. To eliminate this problem, the current and frequency were reduced from 300  $\mu$ A and 50 Hz (total charge=.6  $\mu$ C) to 250  $\mu$ A and 33 Hz respectively (total charge=.33  $\mu$ C); these parameters were used in all subsequent trials (2-13).

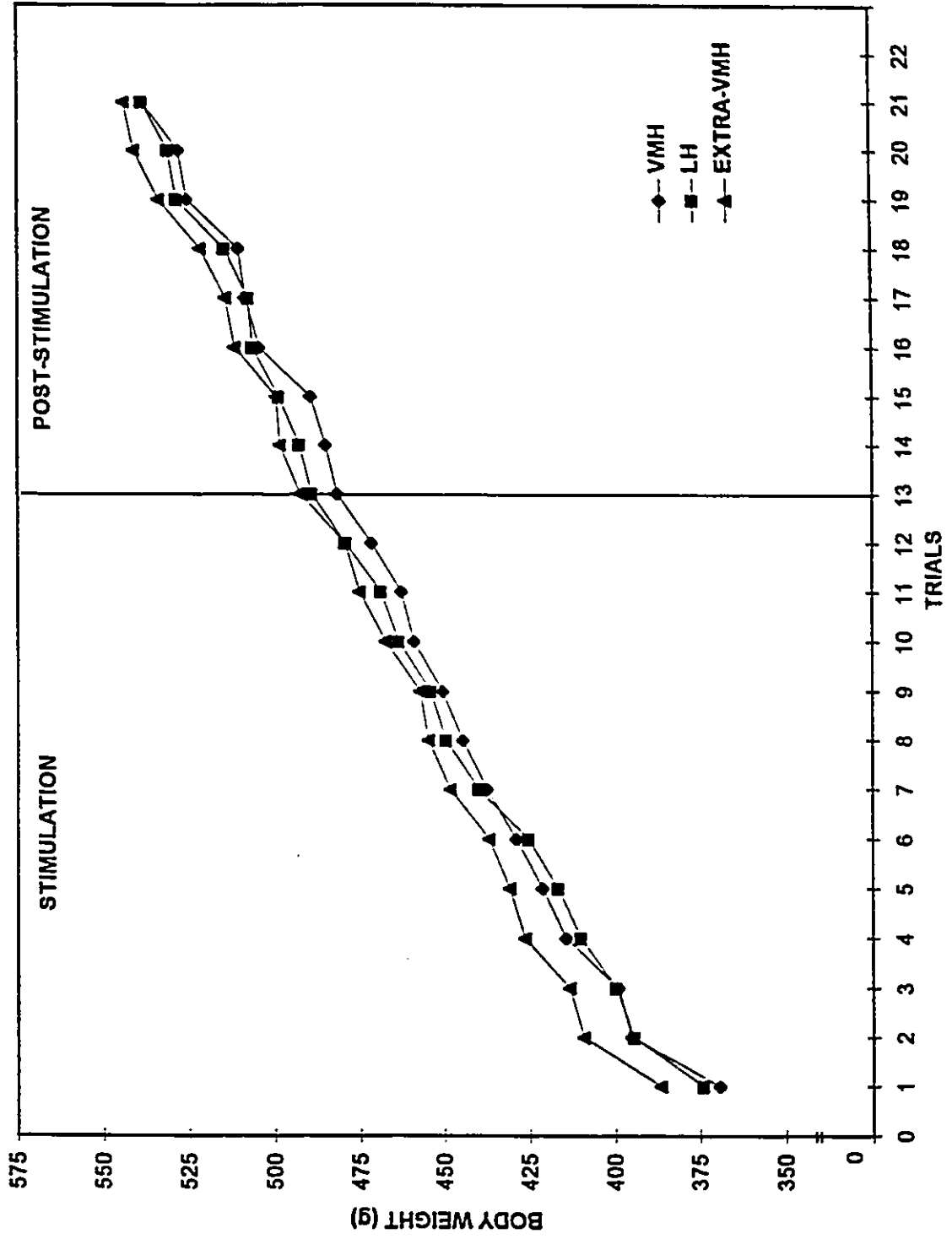
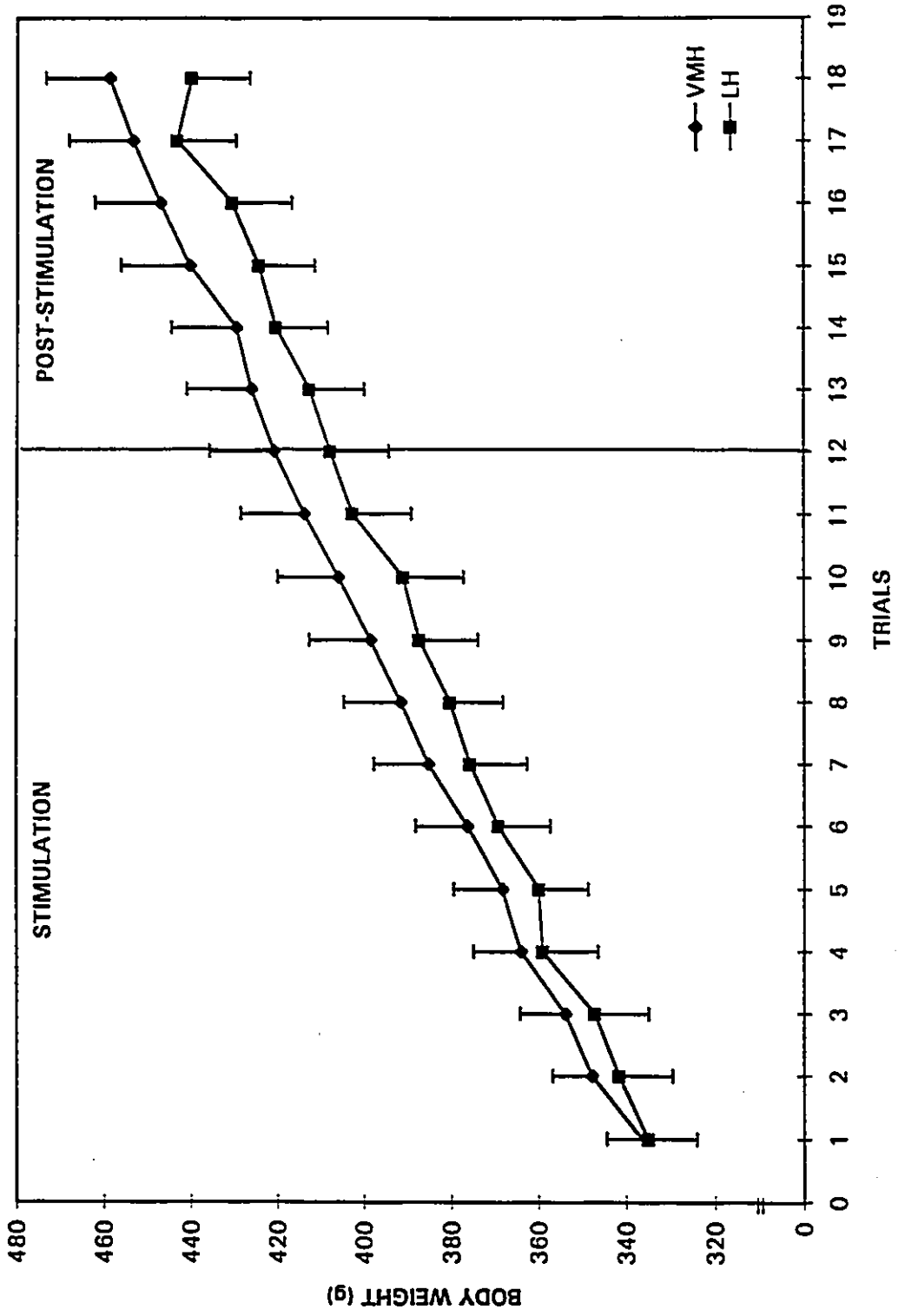


TABLE 2

## Mean Epididymal Fat Pad Weights

Group	Weight (g)	SEM	n
VMH	9.78	1.20	4
LH	10.27	0.85	4
extra-VMH	9.26	0.72	6

Figure 5. Growth curves for two groups of rats (n=5 per group) with electrodes implanted in the VMH or the LH. All groups received low levels of electrical brain stimulation (current = 300  $\mu$ A, frequency = 50 Hz; total charge = .6  $\mu$ C). Each point reflects the mean group weight as a function of the stimulation trial  $\pm$  S.E.M. Stimulation trials 1-12 were administered over 4 weeks, after which rats were monitored for an additional two weeks.



the VMH or the LH. The differences between this and the former group are explained in the next section (technical and methodological considerations). Again the weight gain for both groups was similar and, in fact, it was the control group that showed a greater tendency towards a reduced weight gain. There was some concern as to the appropriateness of the control group in this case because the LH implanted animals were more aroused during the stimulation (showed more locomotor activity) than the VMH group.

Based on the results obtained from these two groups of rats, it was decided that an extra-VMH group (with electrode placements scattered around the perimeter of the VMH) was the most appropriate control because the degree of stimulation induced activity would most closely approximate that induced by VMH stimulation.

#### Technical and Methodological Considerations

It was immediately apparent that an excessive level of activity was often associated with electrical brain stimulation. Therefore, in the first group of rats (data shown in Figure 4), the current and the frequency were reduced from 300  $\mu$ A and 50 Hz to 250  $\mu$ A and 33 Hz respectively, which decreased the total charge of the stimulation from .6 to .33  $\mu$ C, according to the formula:  
 $Q = I \cdot n \cdot d$  where  $Q$  = the charge in  $\mu$ C,  $I$  = the current in  $\mu$ A,

$N$  = the number of pulses in the 400 ms stimulation train, and  $d$  = the pulse duration in  $\mu$ s (Gallistel, 1978).

Decreasing the total charge had the desired effect of reducing the level of stimulation-bound activity. However, it also meant that the parameters employed by Pawson (1988a) were altered, and the fact that a difference was not observed between the stimulated VMH and the two control groups may be, at least in part, attributable to this change in methodology; the effect of decreasing the total charge on the rate of weight gain is unknown. Therefore, in subsequent work, we deemed it prudent to use exactly the same stimulation values as did Pawson (1988a). If stimulation-bound activity was excessive (so extreme that a rat risked injury) it was decided that the animal would be removed from the study. Another change grew out of a consideration of the effect of diurnal cycling on the experimental outcome (Pawson, personal communication). Light onset marks a transitional point in the diurnal cycle of metabolic rate and lipid metabolism; in the rat, metabolic rate decreases dramatically and there is a shift from a lipogenic to a lipolytic metabolism (Le Magnen & Devos, 1970). Previous work has shown that VMH stimulation enhances metabolic rate (Atrens et al., 1985; Pawson et al., 1987). Furthermore, Atrens et al. (1985) showed that this increase is not correlated with changes in respiratory quotient (a measure which indicates the type of fuel, i.e.

carbohydrate or fat, utilized by the animal). Therefore, it was speculated that VMH stimulation commencing at light onset would prevent the decrease in metabolic rate but would not interfere with the shift in metabolism from fat storage to fat utilization. A pairing of high metabolic rate with lipolytic activity should be optimal in inhibiting weight gain; this was the rationale behind the decision to begin testing as close to the end of the rats' active phase as possible. In addition, it was surmised that the age of the rats could be an important factor because the effects of VMH stimulation might be more potent if the animals were showing a high rate of weight gain or in other words growth. For this reason, the second group of animals (data shown in Figure 5) were younger than the first group. However, they developed severe post-surgery infections, requiring chronic treatment with antibiotics. There was some concern that the extended antibiotic treatment, which might have interfered with liver function and thus altered normal metabolism, affected their rate of weight gain.

The pilot work, in its entirety, highlights a few factors which should be considered when studying the effects of chronic VMH stimulation. The first, and most critical, is the need for a stimulated experimental control group. The results show that electrical brain stimulation in general leads to increased arousal and locomotor activity in the rats. In addition, due to the central role of the liver

in energy metabolism, substances which affect liver function (such as antibiotics) should be avoided. Other considerations, which may be important to maximize the effects of VMH stimulation, include the time of testing and the age of the animal.

### EXPERIMENT 1

The objective of this study was to examine weight gain in three groups of rats; one that received VMH stimulation, one that received stimulation to areas adjacent to the VMH (extra-VMH group), and another that was unstimulated (VMH implanted control). The latter was similar to the control group used by Pawson (1988a). It is suggested that the stimulated control group permits an estimate of the magnitude of the experimental effect (i.e. reduced weight gain) that is due to the non-specific effects of electrical brain stimulation, such as increases in arousal and motor activity; assuming that these factors are more or less equal across sites. In addition, it has been shown that stimulation of other hypothalamic areas elicits an increase in metabolic rate to varying degrees (Pawson et al., 1987). Therefore, stimulation of brain areas other than the anterior VMH may result in a significant inhibition in

weight gain due to changes in both metabolic rate and stimulation-induced activity. This study was designed to address some of these issues, and specifically to examine the degree to which the reduced weight gain could be attributed solely to VMH stimulation.

### Method

#### *Subjects and Surgery*

Thirty-eight male Long-Evans rats (Charles River, Quebec) weighing 225-250 g on arrival were housed separately in plastic cages and allowed free access to tap water and standard rat chow. They were maintained on a 12 hr light and 12 hr dark cycle with light onset at 0700 hrs. Food intake and body weight were monitored for one week prior to surgery, after which the animals were assigned to one of three groups - VMH stimulated, extra-VMH stimulated, and VMH implanted control. The rats' weights at the time of surgery ranged from 279-314 g.

Each rat was anaesthetized with 65 mg/kg ip of sodium pentobarbital (Somnotol). A subcutaneous injection of atropine sulfate was administered approximately 15 minutes prior to surgery to minimize mucus production in the animal's respiratory passages.

Surgery was conducted using standard stereotaxic procedures. A single electrode was aimed at the right or

left VMH, or at the tuber cinereum just anterior and lateral to the VMH and ventral to the LH. The flat-skull coordinates were based on the Paxinos and Watson (1986) atlas. They were 2.3 mm behind bregma, 0.6 mm lateral to the mid-sagittal suture, and 9.5 mm below dura for the VMH. Coordinates for the tuber cinereum were 1.8 mm behind bregma, 1.0 mm lateral to the mid-sagittal suture, and 9.4 mm below dura.

The electrodes were prepared from lengths of 0.25 mm stainless steel wire insulated with EpoxyLite to the flattened tips. A flexible stainless steel wire wrapped around each of four stainless steel skull screws served as the current return. The entire assembly was secured to the skull with dental cement.

#### **Apparatus**

The testing chambers were 27 cm long x 25 cm wide x 30 cm tall and were constructed of Plexiglas fronts and backs and perforated stainless steel sides. Stimulation was provided by constant current amplifiers (Mundl, 1980) and integrated circuit pulse generators, built in house. The current was monitored on an oscilloscope by reading the voltage drop across a 1 K $\Omega$  precision resistor in series with the rat. To avoid charge accumulation at the electrode tip, the current was shunted to ground between pulses via a low resistance path.

### **Testing**

Animals had a minimum four day recovery period following surgery. Stimulation sessions started within 90 minutes of light onset, after the weights and food intake had been recorded. Each session lasted three hours and comprised 20 equally spaced 60 second trials, that is, 1 trial every 9 minutes. Stimulation trains within each trial were cycled in a 400 ms on and 600 ms off pattern. Each train consisted of 300  $\mu$ A square wave cathodal pulses 100  $\mu$ s in duration, delivered at a frequency of 50 Hz.

### **Histology**

After a 10 day post-stimulation monitoring period the animals were given a lethal dose of sodium pentobarbital and their bilateral epididymal fat pads were removed and weighed. They were then perfused intracardially with a solution of 0.9% saline followed by 10% formalin. The brains were removed and stored in 10% formalin for at least one day and then sectioned at a thickness of 40  $\mu$ m and stained with cresyl violet. The Paxinos and Watson (1986) atlas was used to locate the electrode tips.

Of the 38 rats implanted, 17 were used in the data analysis - five VMH stimulated, seven extra-VMH stimulated, and five VMH unstimulated controls. Five rats were removed because they developed seizures, two were removed due to excessive activity, three pulled their caps, the histology was lost for one animal, and 10 surgeries were considered to

be outside the target area. In the latter instance, the electrode tip lay outside of the VMH in four animals assigned to the VMH implanted control group, two electrode tips penetrated the ventral surface of the brain, and another two pierced the third ventricle. Finally, in the extra-VMH group, the location of the electrode tip was too posterior (3.60 mm behind bregma) in one rat and too dorsal (in the ventrolateral thalamic nucleus) in another animal.

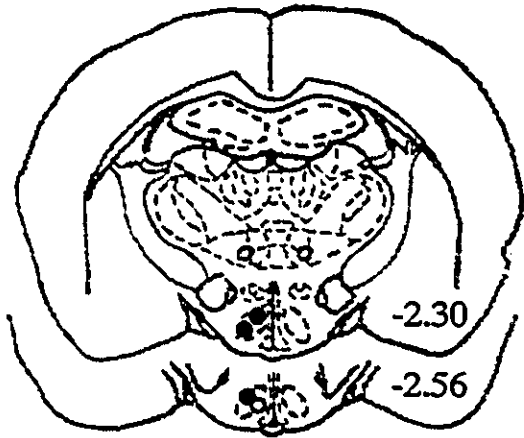
### Results

The placement of the electrode tips is shown in Figure 6. In the two VMH groups, VMH stimulated and implanted control, 9 of the 10 rats had electrodes located within the limits of the anterior to midventromedial nucleus. One of the control animals had an electrode placement bordering the lateral perimeter of the VMH. The electrode placements for the extra-VMH stimulated group were located mainly in the tuber cinereum; two were situated more rostrally, one at the border of the optic chiasm and the other in the suprachiasmatic nucleus.

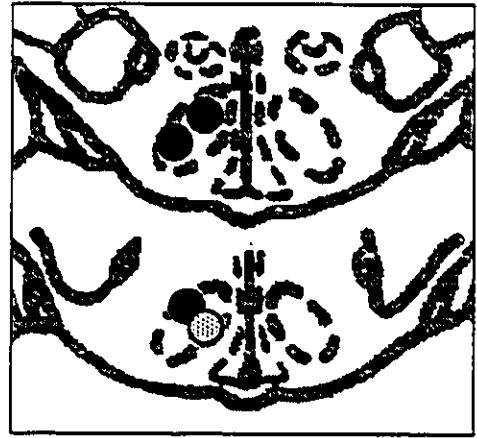
The growth curves for the three groups of rats are shown in Figure 7. The SPSS regression module (SPSS, 1990) was used to perform a multiple regression to ascertain if the data could be best described by a single regression line, or if more variance was accounted for by fitting

Figure 6. Tracings from the atlas plates (Paxinos & Watson, 1986) that best correspond to the location of the electrode tips. The left side of the figure shows a coronal view of the brain with the anteroposterior distance behind bregma indicated in the lower right corner of each section; an enlargement of the hypothalamic area is shown at the right. Closed circles show the placement of a single electrode tip. A stippled circle represents the location of two electrode tips.

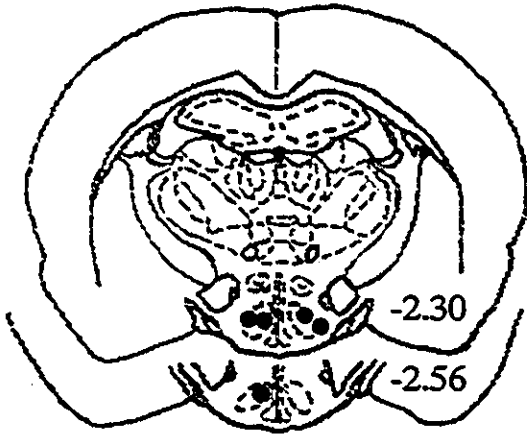
**VMH STIMULATED**



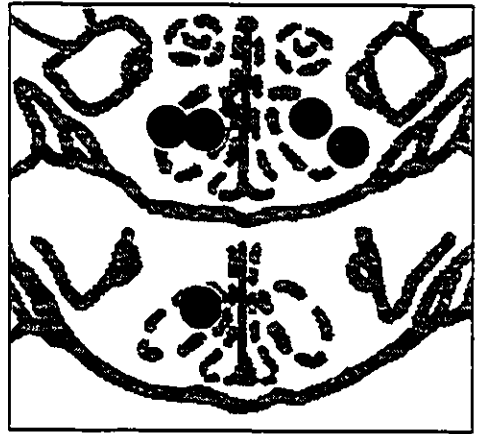
n=5



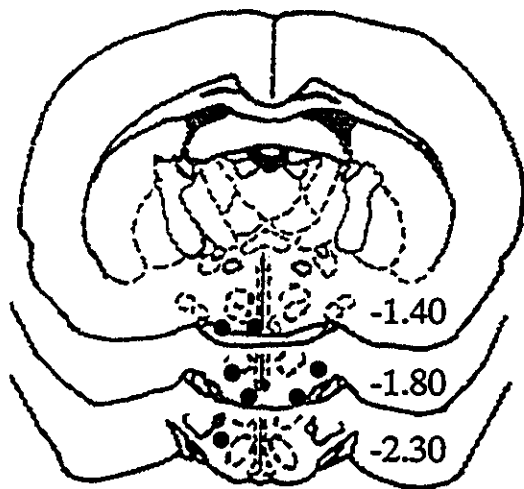
**VMH IMPLANTED CONTROL**



n=5



**EXTRA-VMH STIMULATED**



n=7

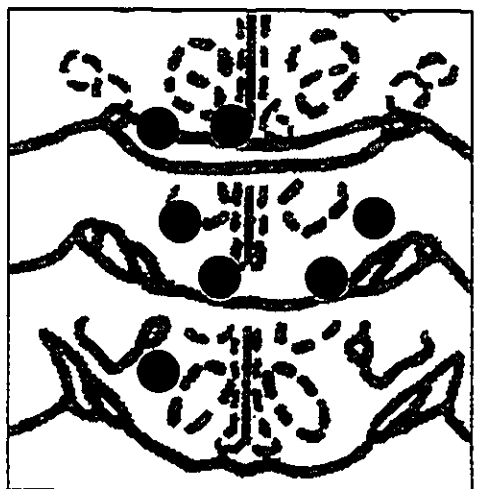
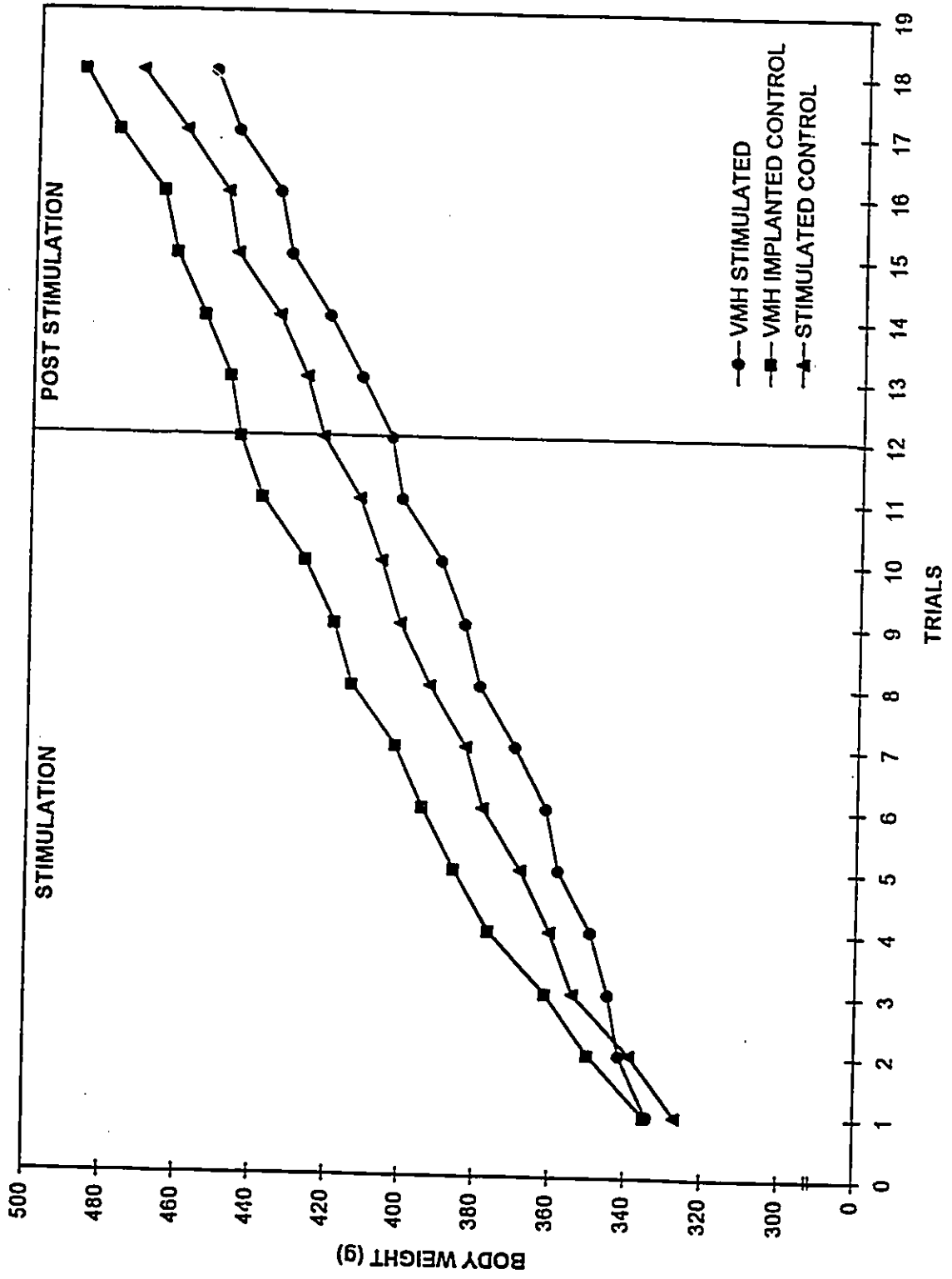


Figure 7. Growth curves for three groups of rats: VMH-stimulated, VMH-implanted control, and stimulated extra-VMH control. There were five animals in each of the VMH and implanted-VMH groups, and 7 in the extra-VMH group. Each point represents the mean group weight. Stimulation trials 1-12 were administered over four weeks, after which animals were monitored for an additional 13 days. For ease of visual examination, error bars were not plotted. The standard error of the mean did not exceed 4.5% in any of the three groups.



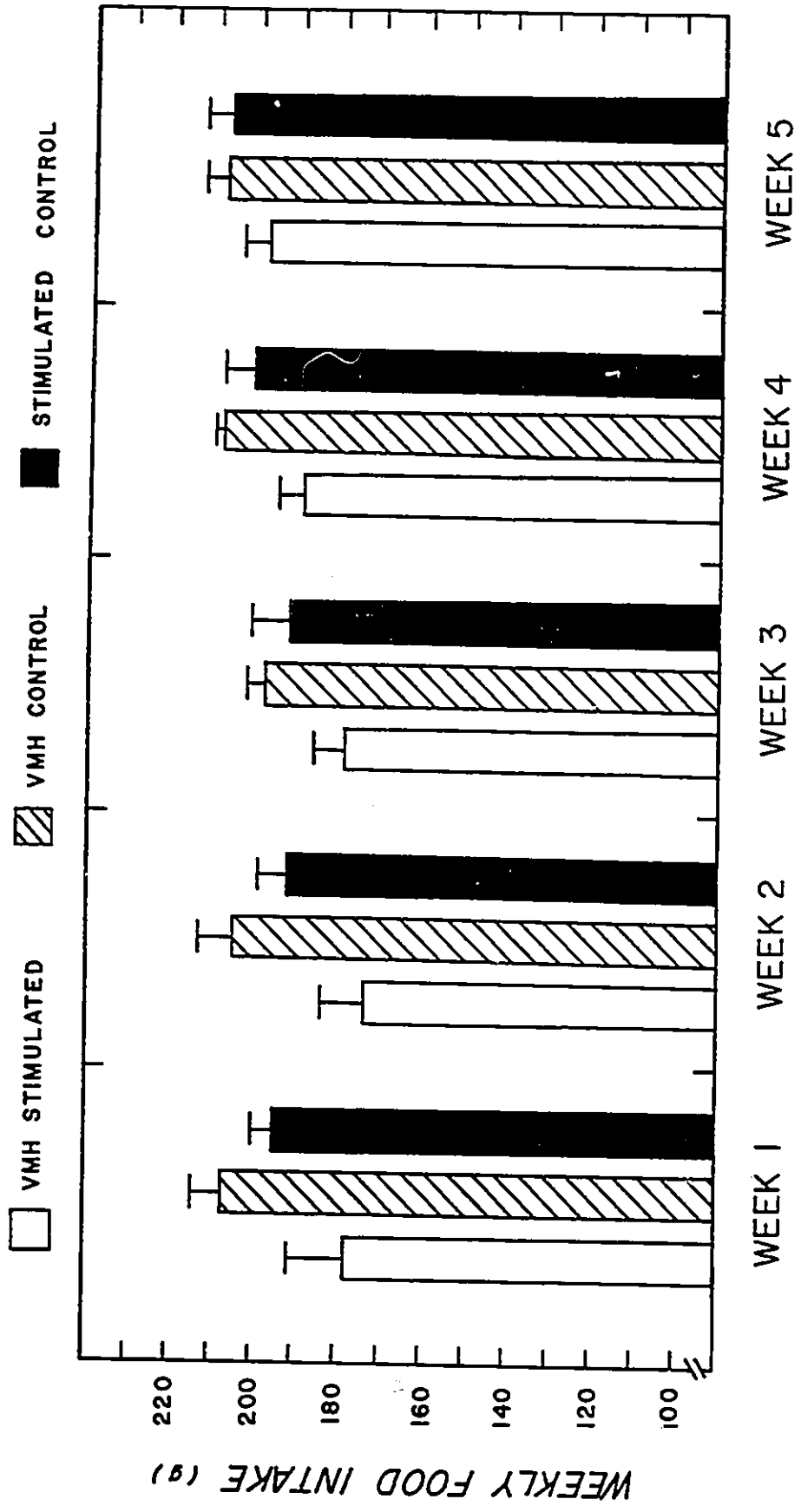
separate regression lines to the three curves. However, before performing the analysis Cochran's test statistic was calculated to determine if the variances of the three groups were similar and thus could be pooled. An alpha level of .05 was used for all statistical tests. Cochran's test statistic showed that the variances of the three groups were similar [ $C(3,6)=.51$ ,  $p<0.5$ ] indicating that the regression analysis was appropriate. Two separate analyses were carried out; one that considered the stimulation phase (trials 1-12) of the experiment and a second that examined both the stimulation and follow-up periods (trials 1-18). In the former case, the analysis showed that the proportion of variance explained by the simple regression relating trial (i.e. time) to weight gain was 64% [ $F(1,219)=389.04$ ,  $p<0.0001$ ]. The next stage of the analysis examined the contribution of the dummy variables pertaining to group; two dummy variables were created because there are three groups (VMH, extra-VMH, and implanted VMH). The interaction between the continuous variable (trial) and the categorical variable (group) was formed by multiplying trial by each of the dummy variables comprising group (Aiken & West, 1991); hence, there were also two interaction terms. The addition of these terms (group and interaction) was found to contribute significantly to the regression model predicting weight gain ( $F$  change=19.21,  $p<0.0001$ ;  $R^2$  change=.09). This means that the data are better represented by three (rather

than one) regression lines, and that the variance explained by the group and interaction terms is 9%. The test for parallelism, which determines if there is an equivalent incremental difference in the weight gain of rats in all three groups (regardless of trial), revealed a significant interaction ( $F$  change=4.49,  $p$ =.012). This indicates that at least two of the three fitted regression equations have significantly different slopes (rate of weight gain). However, it should be noted that the value for  $R^2$  change, although statistically significant, is only 1.11%. Further analysis revealed that a significant difference in slope was only observed between the VMH implanted and VMH stimulated groups ( $F$  change=8.94,  $p$ =.003). A Bonferroni correction for multiple comparisons was applied to this, and all subsequent, pairwise comparisons ( $\alpha$ =.05/3). The slopes for the VMH-stimulated and extra-VMH stimulated groups were similar, as were the slopes for the two control groups (extra-VMH and implanted VMH).

When both the follow-up and stimulation periods were included in the analysis, the results again showed that the data are best represented by three separate regression lines. However, a significant interaction between group and time was not observed.

Figure 8 shows the weekly food intake for the three groups during the four week stimulation and one week post-stimulation periods. A two-way analysis of variance with

Figure 8. Total weekly food intake for the three groups of rats during the four weeks of stimulation and one week post-stimulation. Data shown are group means  $\pm$  S.E.M.



repeated measures on the factor time was performed on the data collected during the stimulation period using the BMDP4V statistical package. The follow-up week was not included in the analysis because differences in food eaten were most apparent during the stimulation phase. It follows that if a real difference in food intake existed among the groups, then it should be revealed during the stimulation phase. No significant difference was found among the groups when food intake was collapsed over the entire stimulation period [ $F(2,14)=3.72$ ,  $p=0.051$ ]. The Greenhouse-Geisser adjusted F value for the factor time just reached significance,  $F(2.28,31.87)=3.20$ ,  $p=0.048$ , indicating that there was a statistically significant change in food intake over the four week period for all rats. No significant interaction effect between group and week was observed [ $F_{\text{adjusted}}(4.55,31.87)=0.80$ ,  $p=0.550$ ].

The efficiency of food utilization, defined as the ratio of weight gain to food intake (Bernardis & Bellinger, 1979; Boyle et al., 1978; Hoover-Plow & Clifford, 1978; Keesey et al., 1978) was determined for each group over the stimulation period. Examination of weekly values of percent efficiency of food utilization (see Table 3) clearly shows that there was a decrease in this estimate for the VMH stimulated group during the first week of stimulation relative to the two control groups,  $F(2,14)=17.51$ ,  $p<0.01$ . Pairwise comparisons using the Tukey-Kramer modification of

**TABLE 3**  
Percent Efficiency

Week	Group		
	VMH Stimulated	VMH Implanted	Stimulated Control
1	8.6* (0.9)	20.3 (0.9)	17.0 (1.9)
2	11.6 (1.5)	12.4 (0.8)	11.8 (0.7)
3	11.1 (2.1)	12.6 (1.6)	11.9 (0.9)
4	11.7 (0.8)	9.8 (0.7)	10.2 (0.5)

Note. The efficiency ( $\pm$  SEM) of the VMH Stimulated group during week 1 was significantly less than the efficiency of the two control groups ( $p < 0.01$ ). This difference disappeared by the second week of stimulation.

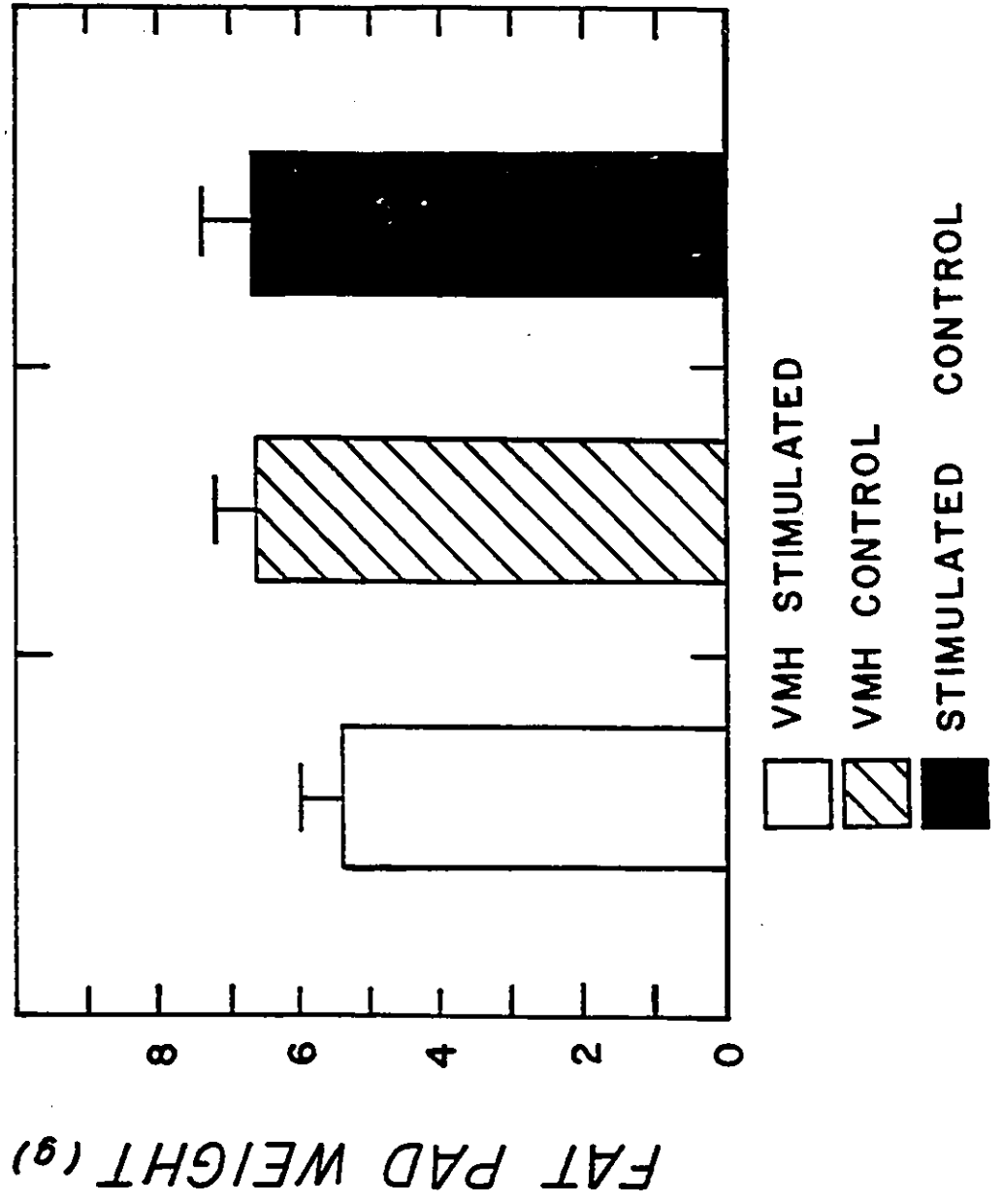
the HSD (honestly significant difference) test revealed a significant difference between the VMH-stimulated group and each of the control groups ( $p < 0.01$ ), whereas the control groups did not significantly differ from each other.

Figure 9 shows the mean bilateral epididymal fat pad weight for the VMH-stimulated group was less than that of either control group, however, an ANOVA showed this difference to be nonsignificant  $F(2,14) = 1.63$ ,  $p > 0.05$ .

### Discussion

There is good reason to suggest that a portion of the reduced weight gain can be attributed solely to the effects of VMH stimulation. First, a visual comparison of the growth curves for the chronically stimulated groups (VMH and extra-VMH) to the VMH implanted control curve (see Figure 7) shows that about 50% of the difference in weight gain can be accounted for by the non-specific effects of electrical brain stimulation, such as increased arousal and locomotor activity. This conclusion is based on the fact that the growth curve for the extra-VMH group lies between the curves for the other two groups. Figure 7 also shows that the reduction in weight gain in the VMH-stimulated group was approximately double that of the extra-VMH group, suggesting that this effect may indeed be site specific. When these

Figure 9. Mean bilateral epididymal fat pad weight  $\pm$  S.E.M. for each group of rats.



results were examined statistically, the fact that the growth curves for the three groups of rats, VMH stimulated, VMH implanted control, and stimulated control, are best represented by three separate regression functions also supports the notion that chronic VMH stimulation expressly inhibits weight gain. When only the stimulation phase was examined, the analysis shows that VMH stimulation specifically inhibited weight gain, as demonstrated by the significant difference between the slopes of the growth curves for the VMH stimulated and implanted control groups. When the follow-up period was included in the analysis this effect disappears; the lines are, at least from a statistical perspective, parallel. The fact that the proportion of variance explained by the interaction between trial and group is small (1.11%) may account for this outcome. This is not to imply that the interaction term is trivial; on the contrary, theoretically speaking it is the proportion of variance explained by the addition of the dummy variables (9%) that is of interest to us. The fact that trial (i.e. time) accounts for 64% of the variance is hardly surprising given that the rats were immature when they entered the experiment. Therefore, it is suggested that the addition of data from the follow-up period "washes out" the relatively small interaction effect. Nonetheless, the results, when taken together and considered within the theoretical framework of the study, suggest some aspect of

VMH stimulation in particular contributes to an inhibition of weight gain. Moreover, the outcome of this chronic study is consistent with the results following acute VMH stimulation. In the latter, increases in metabolic rate elicited by acute VMH stimulation are approximately halved when motor activity is abolished by an injection of Rompun (Pawson et al., 1987). Therefore, an intriguing possibility is that the difference in the rate of weight gain between the two stimulated groups (VMH and extra-VMH) is due to an increase in metabolic rate, which is independent of increases in motor activity, brought on specifically by stimulation of the VMH. The exact cause(s) of the reduced weight gain are unknown. If the effect is due to a transitory increase in arousal and activity, as seen in animals receiving electrical brain stimulation, then the reduction should be temporary. If such were the case, it would be expected that the difference in the slopes of the growth curves between the two stimulated groups and the VMH implanted control group would disappear during the post-stimulation period, and that the stimulated groups would catch up with the sedentary group in weight gain. Alternatively, if VMH stimulation leads to a permanent reduction in the rate of weight gain, then the difference in slopes between the VMH stimulated and the two control groups would be expected to endure. The length of the follow-up period in this study was insufficient to resolve this issue;

however, Pawson (1988a) did find that the weights of VMH stimulated rats remained significantly lower than unstimulated controls at least one month post-stimulation.

A second factor contributing to the reduced weight gain may be the decrease in food intake for the VMH group over the stimulation phase (weeks 1-4). However, an analysis of these data showed that there was no difference among the groups with respect to amount eaten, nor was there any interaction effect; only the main effect of time was significant. The outcome of this analysis is consistent with the findings of Pawson (1988a), who reported that rats receiving VMH stimulation ate the same amount of food as control animals but showed a reduced net weight gain. Nevertheless, examination of Figure 8 leaves little doubt that VMH-stimulated rats ate consistently less throughout the five week experimental period. The reduction in food intake for the VMH group was most obvious during the first two weeks of stimulation; by the third week, the amount eaten began to approach control levels, and by the end of the post-stimulation period it was similar to that of the control groups. Insight into this phenomenon may be gained by considering the results of acute studies. According to Le Magnen (1983), it is classically accepted that electrical stimulation of the VMH blocks the feeding response in hungry animals; however, the decrease in food intake occurs only during the stimulation trials and is subsequently

compensated by an increase in food consumption. It has been suggested that electrical stimulation of the VMH reduces food intake because it induces lipolysis, which in turn causes hypophagia; this effect may be the basis for the enduring misconception of the VMH as a "satiety centre" (Le Magnen, 1985, 1992). It is possible that chronic VMH stimulation in rats fed ad libitum may be sufficient to depress food intake in the periods between stimulations, presumably via an increased lipolytic mechanism. In the present case, when stimulation was terminated, lipolysis was reduced and rats returned to a more normal level of food intake.

The fact that the bilateral epididymal fat pad weight of Pawson's (1988b) VMH stimulated group was significantly less than that of his unstimulated group provides further proof that an increase in lipolysis may be a critical factor in a stimulation induced reduction in weight gain. A decrease in the fat pad weights of the VMH stimulated group relative to the other two groups was also observed in the present study, albeit the decrease was not statistically significant. One possible explanation for the discrepancy between these two sets of results may be the criterion that Pawson (1988b) used to assign rats to the VMH stimulated group. Specifically, only those animals that showed a large increase in metabolic rate, as measured by whole body oxygen consumption, were placed in the VMH stimulated group

(Pawson, 1988b). Therefore, his data may represent a smaller subset of VMH stimulated animals than the group of rats in the current study.

The efficiency of food utilization is a simple approach that is used to assess the metabolism of food (Bernardis & Bellinger, 1979; Boyle, Storlien, & Keeseey, 1978; Hoover-Plow & Clifford, 1978; Keeseey, Boyle, & Storlien, 1978). In the present case, efficiency was calculated for each group to determine if VMH stimulation alters the ability to use ingested nutrients. It is clear from the data shown in Table 3 that, during the first week of stimulation, there is a dramatic difference in values for percent efficiency between the VMH stimulated group (8.6%) and the other two groups (VMH implanted control, 20.3%; stimulated control, 17.0%). However, this difference disappeared by the second week of stimulation. The high values obtained for the extra-VMH stimulated and the VMH implanted groups during the first week were also somewhat mystifying because it was expected that the percent efficiency in each of these two groups would be similar over the entire stimulation phase (weeks 1-4). One possible explanation is that these elevated values were a consequence of the surgery. Hoover-Plow and Clifford (1978) examined the metabolic effects of surgical trauma on young rats by comparing the efficiency of food utilization in operated, sham-operated, and control animals. They found that efficiency was significantly

higher in both operated groups, compared to the control group, from postsurgery days 2 to 6. The sham operation, which was performed under ether anaesthesia, involved making a 2-3 cm abdominal incision, lifting the uterine horns through the incision, returning them to the abdominal cavity, and suturing the wound (Hoover-Plow & Clifford, 1978). The trauma caused by this particular sham-surgery is similar to that produced when an electrode is implanted in the VMH; both require that an incision is made but no tissue or organs are removed. Consistent with this idea that both surgical procedures cause a similar level of stress is the fact that Hoover-Plow and Clifford (1978) report that the sham-operated group showed an efficiency of food utilization of 18% during the first week following surgery (post-operation days 1 to 6). This value is very similar to the ones obtained for the implanted and stimulated control groups (20% and 17% respectively) during the first week of stimulation in the present study and may suggest that the rats had not fully recovered from the trauma of surgery. In addition, the 11% efficiency of their unoperated control group during the first week post-surgery (Hoover-Plow & Clifford, 1978) resembles the efficiencies reported in this study for weeks 2-4 for all groups. Hoover-Plow and Clifford (1978) propose that there could be an endogenous metabolic response to trauma that triggers an increase in the efficiency of food utilization; such a mechanism enables

the animal to compensate for the initial weight loss following surgery. It is possible that VMH stimulation disrupts this process and thus interferes with the re-establishment of normal body weight. This theory would explain the low values obtained for the VMH stimulated group during week 1. It appears that the VMH-stimulated group is as efficient at metabolizing food as the two control groups in weeks 2 to 4 because percent efficiency of food utilization is similar for all three groups. This is consistent with the findings of Keesey, Boyle, and Storlien (1978) who report that rats with lateral hypothalamic lesions show a normal efficiency of food utilization but do so at subnormal weight levels. Lesion of the lateral hypothalamus produces an effect similar to stimulation of the VMH in so far as both lead to an increase in the activity of the sympathetic nervous system. Apparently this shift in sympathetic tone does not alter an animal's ability to use ingested nutrients for weight gain; however, it may reduce the set-point for body weight. This would explain the decreased efficiency seen in the VMH stimulated rats during the first week of stimulation; perhaps they did not regain the weight lost due to surgical trauma simply because their body weight set-point had been re-established at a new, and lower, level.

It is likely that both resting metabolic rate and BAT thermogenesis are important factors in the determination of

body weight set-point. For example, it has been reported that VMH stimulation increases blood flow to interscapular BAT, as well as raising heart rate and cardiac output (Iwai, Hell, and Shimazu, 1987). Pawson (1987) has also reported an increased metabolic rate subsequent to VMH stimulation, and others (Kelly & Bielajew, 1991; Perkins et al., 1981; Thornhill & Halvorson, 1990, 1994) have shown that VMH stimulation elicits BAT thermogenesis. Hence these studies suggest a possible mechanism through which VMH stimulation may act to lower the set-point for body weight.

## EXPERIMENT 2

Pawson's (1988a) work shows that repeated VMH stimulation leads to a persistent reduction in weight gain in normophagic rats. The findings of our first study (Stenger, Fournier, & Bielajew, 1991) also suggest that chronic stimulation of this area inhibits weight gain, and that this effect can not be attributed to a decrease in food intake (recall that an analysis of the data on food intake failed to show a significant difference among the VMH stimulated, extra-VMH stimulated, and implanted control groups). If a decrease in food intake doesn't explain the reduction in weight gain, then it may be the result of an elevation in energy expenditure. This has been suggested by several studies that have reported an increase in oxygen consumption due to VMH stimulation (Atrens et al., 1985; Morimoto, Murakami, Ono, Watanabe, & Sakata, 1986; Pawson et al., 1987). Also consistent with the notion that the VMH plays an important role in the modulation of energy expenditure is the fact that we saw a decreased efficiency of food utilization (weight gain/food intake) during the initial week of stimulation in the VMH stimulated group relative to the two control groups (Stenger et al., 1991). What remains to be elucidated is the mediator of this shift in energy balance. One theory is that the VMH is involved in the control of brown adipose tissue (BAT) thermogenesis

and that non-shivering thermogenesis is a factor in the hypermetabolism (Kelly & Bielajew, 1991; Perkins et al., 1981). However, it is also well known that VMH stimulation leads to increased arousal and muscle activity (Atrens et al., 1985; Kumon et al., 1976; Shimazu, 1981). Therefore, one purpose of this second experiment was to evaluate the contribution of BAT thermogenesis and stimulation-bound activity to the stimulation induced reduction in weight gain. The decrease in the efficiency of food utilization and the inhibition of weight gain during the first week of stimulation were also examined in more detail. Given the outcome of the first study (Stenger et al., 1991) it was suspected that the initial week of stimulation (three stimulation sessions) might be sufficient to produce the reduced weight gain. In addition, the results of the first experiment showed that an extra-VMH stimulated group is the most appropriate control group for this type of work because activity accounted for approximately 50% of the effect. Therefore, only two groups of rats (VMH and extra-VMH) were examined in the present experiment; an implanted control was not included. This time the electrode placements of the extra-VMH group were more widely scattered than in previous work because it was speculated that fibers of passage, as well as cells within the VMH, may contribute to the reduction in weight gain.

## Method

### *Subjects and surgery*

Forty-nine male Long-Evans rats, weighing 225-250 g on arrival, were used in this study. They were maintained on a 12 hr light and 12 hr dark cycle with light onset at either 0700 or 0900 hrs. The housing and treatment of the animals was the same as that described in Experiment 1. The weights at the time of surgery ranged from 292-330 g. A single electrode was aimed at the VMH or the surrounding area. The coordinates for the VMH were similar to those used in the previous study; 2.3-2.56 posterior to bregma, 0.5 lateral to the midsagittal suture, and 9.5 below dura. Coordinates for the extra-VMH group were 2.8-3.14 behind bregma, 1.0-1.5 lateral to the midsagittal suture, and 9.2-9.8 below dura.

### *Apparatus and Testing*

The rats received three stimulation sessions (one session every other day for a week) followed by a four week monitoring period in which food intake and body weight were recorded three times per week. The apparatus and the testing procedure for the stimulation phase was the same as that described in Experiment 1. During the first stimulation session, the stimulation bound activity of each rat was categorized on a two point scale, similar to the one used by Atrens et al. (1985). A score of 0 included

behaviours ranging from a position change to walking, rearing, and sniffing. Extreme activity was assigned a value of 1 and was defined as running, climbing, and jumping.

Just before sacrifice, each rat was anaesthetized with sodium pentobarbitol (65 mg/kg ip) and an incision was made exposing the interscapular BAT pad. A thermocouple thermoprobe (Mini-Mitter, Inc.) was placed between the shoulder muscle and the BAT pad. A rectal thermometer monitored core body temperature which, for 24 of the 33 rats was maintained using a homeothermic blanket unit (Harvard Scientific Ltd.); core temperature was not systematically monitored in the other nine animals. In addition, the baseline of BAT temperature was less rigorously tracked in these animals than in the other 24 rats. In the latter group, the baseline was considered stable if there was a maximum variation of 0.3°C in BAT over a 20 minute period (for 22 of the 24 rats the change in baseline was 0.2°C or less). Once a stable baseline had been reached, each rat received one 60 s trial of stimulation, comprising 100  $\mu$ s square wave cathodal pulses. The pulse amplitude and current were set at 300  $\mu$ A and 50 Hz, respectively; the total charge was therefore 1.5  $\mu$ C. Changes in BAT and core temperatures were recorded for a minimum of 20 minutes after the stimulation, and the criterion for a positive BAT response was an increase of at least 0.4°C within this time

period, similar to that employed in other studies of this nature (Kelly & Bielajew, 1991, 1995).

### ***Histology***

Perfusions and histology were carried out as described in Experiment 1. The analysis was conducted on data from 33 of 49 rats. Three rats were removed due to excessive stimulation bound activity, six were removed because they developed seizures, another animal was excluded because it continued to lose weight during the post-surgery recovery period, and the histology was lost for one animal. Finally, based on histological verification, five of the implants were outside of the target area. Specifically, the electrode tip penetrated the ventral surface of the brain in three rats and in another two animals it pierced the third ventricle.

### **Results**

Table 4 shows the mean weight gain and food intake for the two groups of rats (VMH and extra-VMH) for the week preceding surgery. Figure 10 shows the location of the electrode tips for the VMH and extra-VMH groups. The former are mainly clustered in the anterior to midportion of the VMH; two rats had electrode tips in the posterior VMH. The electrode placements of the 16 animals in the extra-VMH group were intentionally more widely scattered; they were

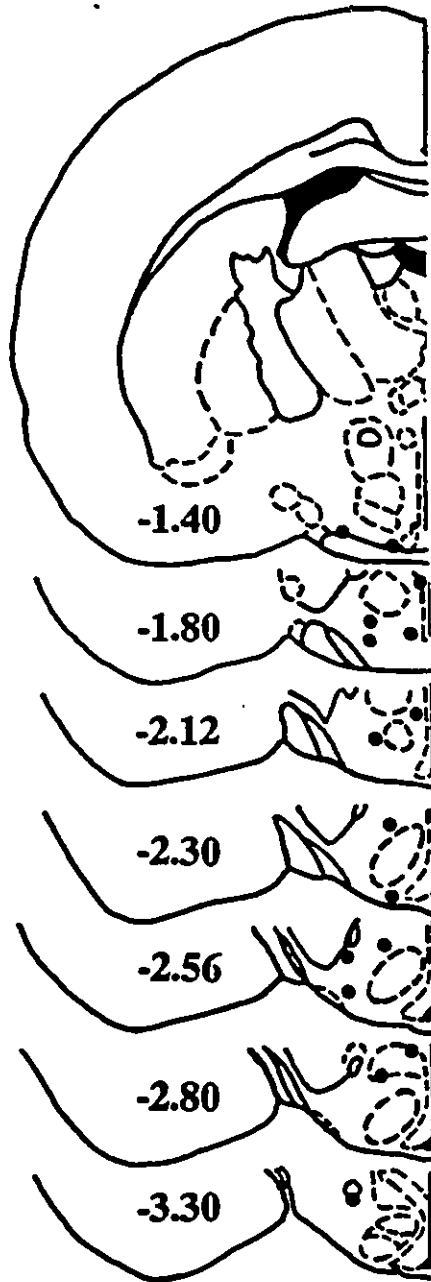
TABLE 4

Mean Weight Gain and Food Intake for the Week Before Surgery

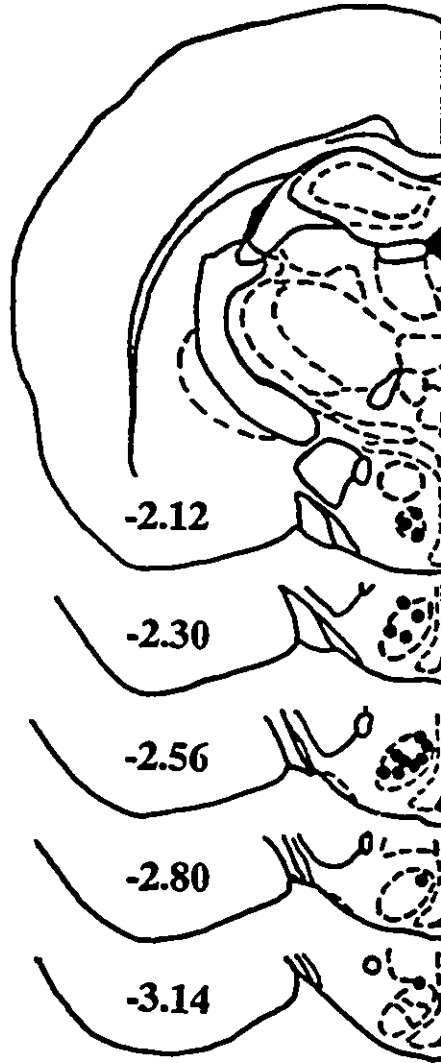
Group	Weight Gain (g)	Food Intake (g)
VMH	53 $\pm$ 2	188 $\pm$ 2
Extra-VMH	51 $\pm$ 2	187 $\pm$ 3

**Figure 10.** Tracings from the atlas plates (Paxinos & Watson, 1986) that best correspond to the location of the electrode tips in extra-VMH and VMH groups. Electrode tips are identified by filled circles. The anteroposterior distance behind bregma is shown in the lower left corner of each section.

# EXTRA-VMH



# VMH



predominantly either rostral to the VMH or adjacent to it in the tuber cinereum. Two of the rats in this group had placements bordering the dorsal medial nucleus, and a third animal had a more posterior placement, which was situated in the tuber cinereum at the ventral boundary of the fornix.

Weight gain, food intake, and percent efficiency of food utilization are plotted separately in Figure 11. A third group (VMH<sub>g</sub>), which consists of VMH animals minus the two rats with the most posterior placements, has been included in this figure. Panel A shows that VMH stimulation specifically inhibits weight gain. The follow-up period (weeks 1 to 4) reveals that the reduced weight gain is not maintained once stimulation is terminated. In contrast, Panel B shows that the decreased food intake observed during the stimulation week was maintained during the post-stimulation period. The percent efficiency of food utilization (the ratio of mean weight gain to mean food intake multiplied by 100) was reduced in both VMH groups during the week of stimulation but similar to the extra-VMH animals in the post-stimulation period (see Panel C).

Figure 12 shows the growth curves for the VMH and extra-VMH groups. Trials 1 to 4 indicate mean group weights for the time period between the animals' arrival and their surgeries (presurgery phase). The arrows designate the test days (trials 5, 6, 7), that is days on which rats received electrical stimulation, and trial 5 shows the mean weight

Figure 11. Weight gain, food intake, and percent efficiency of food utilization for the extra-VMH and two VMH groups; a VMH group that includes all animals with electrode placements in the VMH, and a VMH subgroup (VMH<sub>g</sub>) in which the data from the two animals with the most posterior VMH placements have been removed. Data shown are for the week of stimulation and the four weeks post-stimulation. The results are expressed as group means  $\pm$  S.E.M.

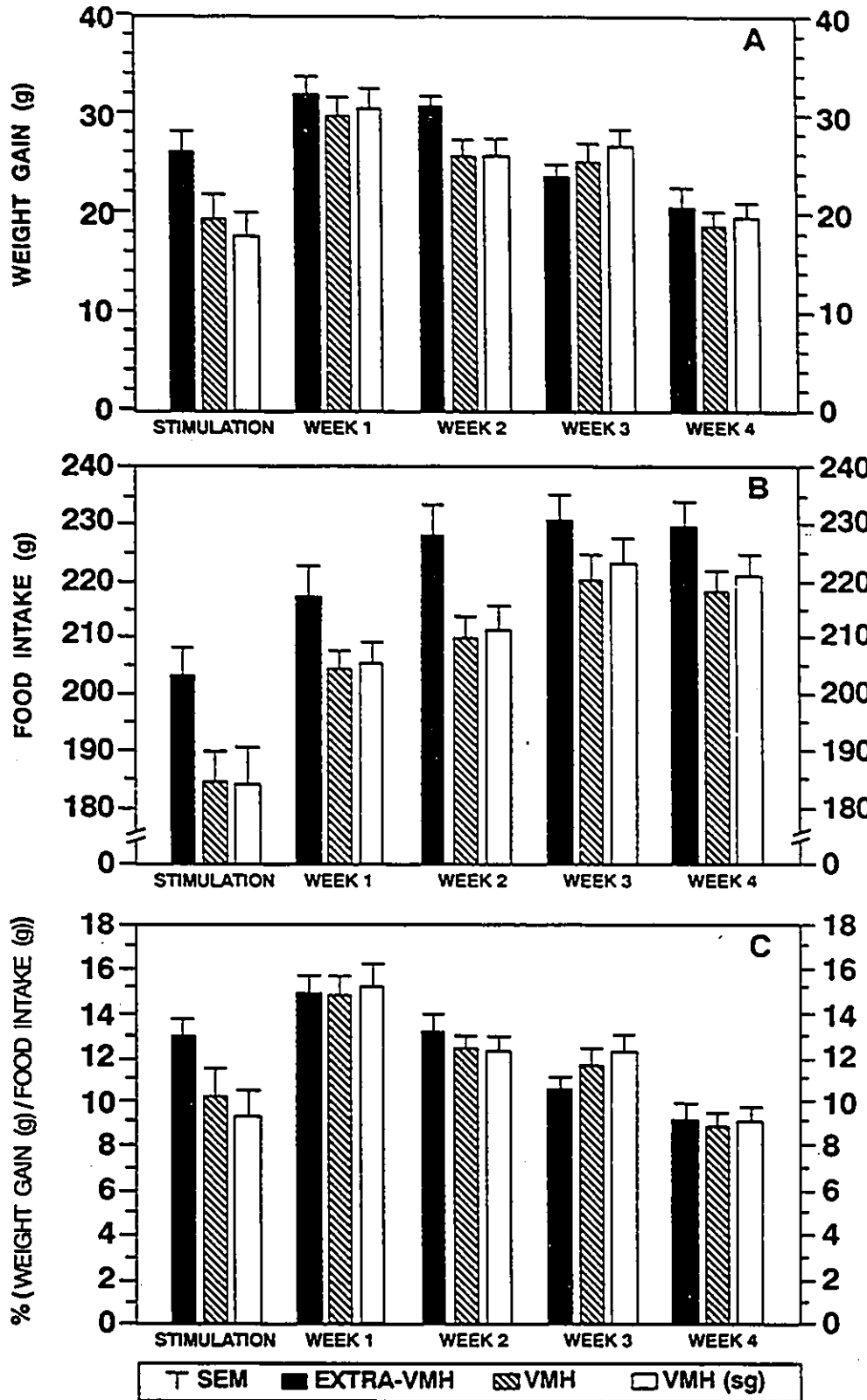
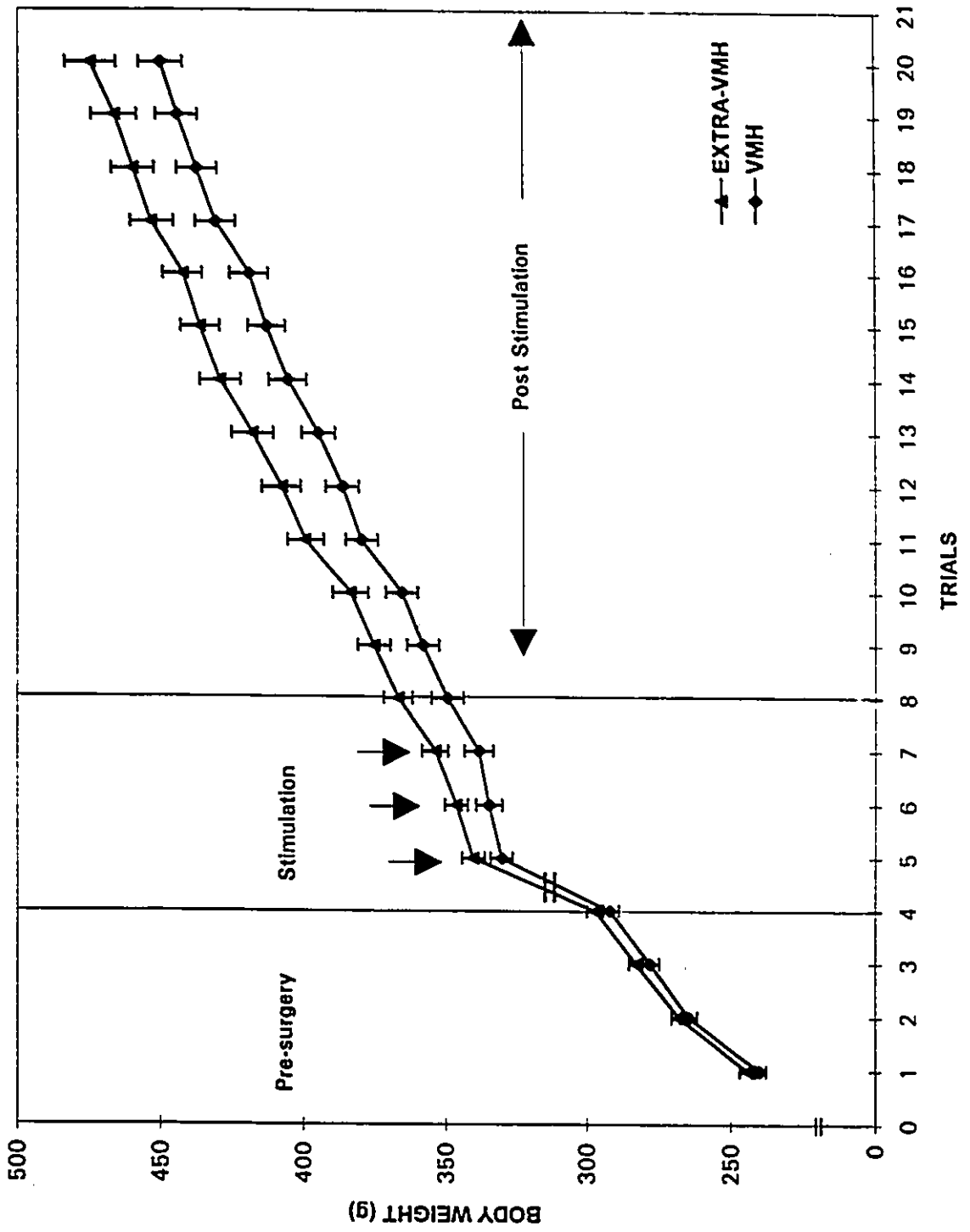


Figure 12. Growth curves for the VMH and extra-VMH groups. Each point depicts mean group weight as a function of test day  $\pm$  S.E.M. The first four data points represents the presurgery phase. The break in the graph between trials 4 and 5 represents the post-surgery recovery period. Three stimulation sessions were administered over a one week period and are indicated by the three arrows. Animals were monitored for four weeks following the completion of the stimulation.



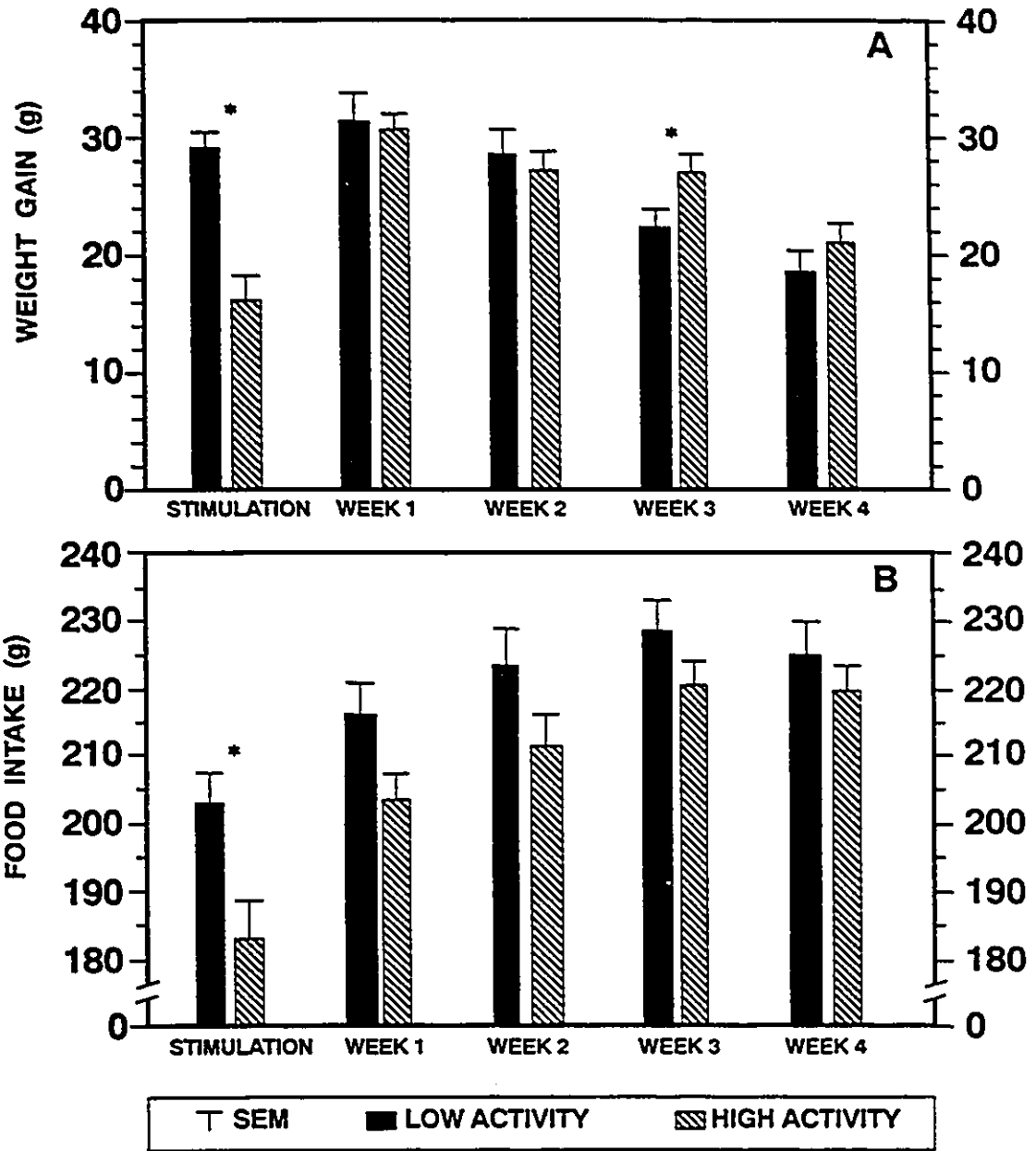
for each group immediately prior to the first stimulation period. Trial 8 marks the beginning of the post-stimulation period and shows the mean weight for each group at the completion of three stimulation sessions. As described previously, a multiple regression model (Berenson et al., 1983) was used to analyze the data. An alpha level of .05 was used for all statistical tests. Cochran's test statistic showed that the assumption of homoscedasticity for the two groups was met [ $C(2,16) = .53$   $p < 0.5$ ] and the variances could be pooled for the regression analysis. Initially two separate analyses were performed; one on the presurgery phase (trials 1-4), and another on the stimulation and follow-up periods (trials 5-20). In the former case the analysis showed that 72% of the variance is accounted for by the simple regression relating time to weight gain. The addition of the group variable (using dummy coding) and the interaction term did not significantly improve predicted weight gain. This suggests that there is no difference between the perspective groups (VMH and extra-VMH) before surgery and the data are best represented by a single regression line.

The analysis of the growth curves from trial 5 to trial 20 inclusive (stimulation and post-stimulation periods) showed that group is a significant contributor to the regression model predicting weight gain ( $F$  change = 39.54,  $p < .0001$ ;  $R^2$  change = .04). This means that, after surgery,

the data are best described by two regression lines - one representing the VMH group, and another, the extra-VMH group. Further analysis showed that the slopes for the two lines were similar, suggesting that the rate of weight gain for the two groups were comparable and that any differences between them were due to dissimilarities that existed since surgery (and not to the stimulation per se). Subsequent to this analysis the stimulation period (trials 5-8) was examined because the suspicion was, if there was a difference in the rate of weight gain between the groups, it would be most apparent during the time when the rats were receiving the stimulation. The outcome of this analysis showed that the groups were significantly different ( $F$  change=8.54,  $p$ =.0003;  $R^2$  change=.10) but that the slopes of the two regression lines were similar; this confirms the notion that any deviation from parallelism was due to group differences that emerged after surgery.

The data from Figure 11 have been replotted in Figure 13; however, in this case group assignment was based on each rat's observed level of stimulation-bound activity rather than electrode placement. Panel A shows that the rats categorized as being the most active had a much lower mean weight gain relative to the less active group during the week of stimulation; this difference disappeared in the follow-up period. Panel B shows that, in general, the high activity group ate less than the low activity group and that

**Figure 13.** Weight gain and food intake for high and low activity groups during the week of stimulation and the four week post-stimulation period. The results are expressed as group means  $\pm$  S.E.M.



this effect was most pronounced during the week of stimulation.

The design of this study is statistically appropriate for a doubly multivariate repeated measures analysis (SPSS/PC+ Advanced Statistics 4.0, 1990). There are two between factors (electrode placement, activity), two within dependent variables (weight gain, food intake), and five levels of time. The analysis revealed a significant interaction of time and activity (Wilks' Lambda (8,22)=0.33,  $p=.001$ ). The post-hoc analysis showed a significant difference between the low and high activity groups during the week of stimulation (Wilks' Lambda (2,30)=0.53,  $p<.0001$ ), both in weight gain [ $F(1,31)=26.38$ ,  $p<.0001$ ] and food intake [ $F(1,31)=7.27$ ,  $p=.011$ ]. The direction of these differences is apparent in Fig. 13; the high activity group gained less weight and ate less food than the low activity group. A significant difference between these two groups was also observed in the third week post-stimulation (Wilks' Lambda (2,30)=.71,  $p=0.006$ ). However, in this instance, only the difference in weight gain was significant [ $F(1,31)=4.824$ ,  $p=0.036$ ], and it was in the opposite direction to that observed during the week of stimulation.

The effect of VMH stimulation on BAT thermogenesis was examined in 33 rats. A stimulation-induced increase in BAT temperature was observed in only two animals (one from each group, VMH and extra-VMH) when core temperature was

maintained with the homeothermic blanket unit. With respect to the nine rats that were tested without the blanket, five showed an increase in BAT temperature that ranged from 0.3 to 1.0°C (three extra-VMH, two VMH). Another extra-VMH animal showed a decrease of 0.4°C in BAT temperature and three rats (one extra-VMH, two VMH) showed no stimulation-induced changes in BAT thermogenesis.

### Discussion

The results of this study suggest that stimulation bound activity is an important contributing factor to the decreased weight gain and food intake observed during the week of stimulation (see Figure 13), more important, in fact, than electrode placement. The doubly multivariate repeated measures analysis revealed that the high activity group showed a significant reduction in both weight gain and food intake compared to the low activity group. For the most part, the differences between the groups disappeared in the four week follow-up period. The one exception was the third week following the stimulation phase, during which the animals in the high activity group gained significantly more weight than their low activity counterparts; it is unlikely that this result represents a rebound effect since it occurred two weeks after the termination of stimulation.

The finding that weight gain and food intake were not affected by electrode placement was unexpected because VMH stimulation is associated with increases in both metabolic rate and locomotor activity (Atrens et al., 1985; Brown, Hunsperger, & Rosveld, 1969a, 1969b; Krasne, 1963; Nakao, 1958; Pawson et al., 1987), and an inhibition of food intake (Dallman, 1984). In early work, the expression of motor activity was studied as escape or aggressive behaviours (Brown et al., 1969a, 1969b; Krasne, 1963; Nakao, 1958). More recently a technique has been developed that allows the examination of one component of these behaviours, namely running. It involves the injection of the hydrogel of a water absorbent polymer into the VMH (Narita, Nishihara, & Takahashi, 1994; Narita, Yokawa, Nishihara, & Takahashi, 1993; Narita, Nishihara, & Takahashi, Yokawa et al., 1989; Yokawa, Shiota, & Takahashi, 1990). In theory, the polymer disrupts the circuitry of the VMH and causes the running behaviour (Narita et al., 1994, 1993; Yokawa et al., 1989; Yokawa et al., 1990). Using this paradigm, it has been found that injection of bicuculline methiodide (a GABA<sub>A</sub> receptor antagonist) into the VMH elicits running activity (Narita et al., 1993; Yokawa et al., 1990). Based on the fact that GABA receptor antagonism has been shown to induce locomotor activity in brain regions that are considered to be more closely associated with motor function than the VMH, such as the corpus striatum (Jones & Mogenson, 1980),

inferior colliculus (Brandao, Tomaz, Leao Borges, Coimbra, & Bagri, 1988) and the posterior hypothalamus (Shekhar & DiMicco, 1987), it is suggested that the VMH may play a role in the integration of motor activity and energy metabolism (Narita et al., 1993). Indeed, in a subsequent study Narita et al. (1994) propose that kainate type glutaminergic receptors in the VMH are involved in eliciting running activity and simultaneously activate the sympathetic nervous system; the outcome of the latter is an increase in blood glucose. Noradrenergic transmission in the VMH is thought to mediate the stimulatory effect of glutaminergic transmission on the sympathetic nervous system, whereas the GABA system in the VMH suppresses both the activity of the sympathetic nervous system and running behaviour. Vissing, Wallace, Scheurink, Galbo, & Steffen (1988) examined the relationship between VMH activity, running, and substrate mobilization; they speculate that exercise-induced increases in VMH activity may be regulated by the same CNS structures that activate locomotion and substrate mobilization. The implication of these studies, when considered together, is that the VMH plays a central role in both energy metabolism and the mediation of locomotor activity.

Our data concur with the literature in that approximately 70% of the rats in the high activity group also had electrode placements in the VMH. It is therefore surprising that the analysis did not reveal an interaction

between electrode placement and activity. One explanation is that the placement effect was masked by a much stronger activity effect. In accordance with this theory is the fact that the difference in weight gain between VMH and extra-VMH groups reaches significance when activity values are not considered in the analysis. Hence, a more sensitive measure for locomotor activity than that used in the present study may be necessary to reveal an interaction. In an acute study, Pawson et al., (1987) removed the contribution of activity by injecting the rats with a sedative/anaesthetic (Rompun). The result was a reduction in the increase in metabolic rate, which was elicited by stimulation of the VMH, by approximately 50%. This outcome implies that muscle tone and locomotor activity may be important contributing factors to the reduced weight gain observed in chronic studies when the animals are conscious and free to move about (Bielajew et al., 1994; Pawson, 1988; Pawson et al., 1987; Stenger et al., 1991).

Figure 11A shows that the mean difference in weight gain between the VMH and extra-VMH groups is enhanced when the two rats with posterior electrode placements are removed from the VMH group (VMH<sub>pg</sub>). This agrees with the work of Pawson et al. (1987) who report that the greatest increases in metabolic rate occur when the anterior VMH and the anterior hypothalamus are electrically stimulated. It may be the case that stimulation of those areas within the VMH

that are associated with the greatest increases in metabolic rate also maximizes the inhibition in weight gain.

Accordingly, a screening procedure that distinguishes those animals with the largest stimulation-bound increases in oxygen consumption might be pertinent in future work.

The fact that the high activity group showed a significant decrease in food intake compared to the low activity group (see Figure 13) is somewhat surprising. Studies have shown that while forced exercise depresses food intake in male rats, this effect is short lived and is subsequently compensated by an increased intake (Applegate, Upton, & Stern, 1982; Nance, Bromley, Barnard, & Gorski, 1977; Stevenson, Box, Feleki, & Beaton, 1966; Thomas & Miller, 1958). It was anticipated that a similar outcome would be seen in the high activity group during the week of stimulation, in other words, a depressed food intake on test days, and an increased intake on other days. It is apparent that the hypophagia was associated specifically with the stimulation because there was no significant difference in food intake between the groups in the follow-up period. The contribution of VMH stimulation to a reduction in food intake can not be completely ruled out because rats with VMH electrodes made up a large portion (70%) of the high activity group. This may be an important factor given the evidence of a reciprocal relationship between sympathetic activity and food intake (Bray, 1990, 1991; Dallman, 1984;

Sakaguchi, Takahashi, & Bray, 1988).

The analysis of the growth curves for the two groups suggests that any discrepancy between the groups in the rate of weight gain (slope of the growth curve) is due to differences that emerged after surgery, and not to the stimulation per se; statistically, the lines are parallel. This is consistent with our first study (Stenger et al., 1991) because a significant difference in rate of weight gain was only observed between the VMH-implanted control group (which did not receive stimulation) and the VMH group; no significant difference was observed between the VMH and extra-VMH groups. More perplexing is the cause of the dissimilarity between the groups. The regression analysis performed on the presurgery period confirms that the mean weight gain for both groups was virtually identical during this time period. Furthermore, rats that did not gain weight and/or showed a decreased food intake after surgery were excluded from the study (this only happened in one instance).

Of the rats monitored with the homeothermic blanket unit, only one VMH electrode placement (one in which the tip was located in the ventral anterior VMH) was associated with an increase in interscapular BAT thermogenesis. Electrical stimulation of all other areas within the nucleus failed to elicit a BAT response (i.e. neither an increase nor decrease in thermogenesis). Kelly and Bielajew (1991) have used a

similar protocol to show changes in BAT thermogenesis. It is possible that the age of the rats (mature in the present study) as well as the heterogeneity of the VMH may explain the lack of a BAT response. A series of studies has shown that while the thermogenic capacity of BAT (as measured by guanosine diphosphate [GDP] binding) is similar in both young and old female rats, a significant decrease in GDP binding is observed in aged relative to young males. In addition, Rothwell and Stock (1983) have found that there is an age-related decline in the ability of rats to activate BAT thermogenesis in response to a cafeteria diet (stock diet plus a choice of highly palatable food).

Concerning the nine rats that were tested without the homeothermic blanket unit, it is difficult to determine if changes in BAT thermogenesis were due to electrical brain stimulation per se, or to a drop in core temperature elicited by an anaesthetic-induced hypothermia. In this latter situation it is known that BAT serves as a primary heat source (Gordon, 1993; Shimizu & Saito, 1991). Nonetheless, the fact that 6 of 9 animals showed a stimulation induced change in BAT temperature suggests that electrical stimulation of certain brain regions in, or around, the VMH are associated with a BAT response.

A number of studies highlight the heterogeneity of the VMH. At neurochemical and electrophysiological levels, it has been shown that both inhibitory interneurons and the

inhibitory neurotransmitter GABA, are found in the VMH nucleus (Blume, Pittman, & Renaud, 1981; Marchand, Defrance, & Stanley, 1982; Murphy & Renaud, 1968; Narita et al., 1993, 1994; Yokawa et al., 1990). It is also known that adrenoceptors in the VMH are involved in the regulation of blood glucose levels both when the animal is in a resting state, and during exercise (Scheurink & Steffens, 1990; Steffens, Damsa, van der Gugten, & Luiten, 1984). In the latter study it is suggested that three separate neural circuits may be responsible for the increases in plasma free fatty acid, plasma insulin, and blood glucose levels elicited by noradrenergic stimulation of the VMH. Narita et al. (1994) propose that glutaminergic receptors in the VMH are involved in inducing hyper-running activity and simultaneously activating the sympathetic nervous system. The latter results in an increase in blood glucose and provides fuel for working muscles. Thus they believe that the VMH may be the central command center that is responsible for the integration of both exercise and energy metabolism (Narita et al., 1994). The inhibitory (GABA) system in the VMH, on the other hand, suppresses the sympathetic system and the expression of running behaviour (Narita et al., 1994; Yokawa et al., 1990). Vissig et al. (1989) suggest that substrate mobilization during exercise may depend on the interaction of different neuronal activity levels within the VMH itself. Hence, there is a substantial

literature suggesting that the VMH is a complex structure that is implicated in a number of different peripheral responses. Although previous work (Pawson, 1988a; Stenger et al., 1991) supports the notion that VMH stimulation leads to a reduced weight gain, the factors contributing to this effect may be difficult to identify due to the complexity of the nucleus. The main contribution of the present study is the demonstration that motor activity plays a significant role in stimulation-bound decreases in weight gain, and points to the need to include a sensitive measure for both stimulation-bound and spontaneous activity in future work.

**An Appraisal of the Relationship Between Brainstem Electrode Placement, Weight Gain, and Food Intake**

The first two experiments showed that there was a tendency for VMH stimulated rats to gain less weight than animals stimulated in extra-VMH regions (see Figures 7 and 12). This outcome was assessed by using a multidimensional scaling analysis (MDS) to examine the data from both studies. The expectation was that the analysis would provide both an overview of the results and make the data more comprehensible. Unlike more rigorous statistical procedures, MDS provides a way of examining the hidden structure in the data by generating a spatial representation of the results - something akin to a map. Furthermore, it allowed us to evaluate the results within the theoretical framework proposed by the thesis; this is particularly helpful when considering the basic question of whether there is (or is not) a difference in weight gain between extra-VMH and VMH groups. Hence, the data for rats that received VMH or extra-VMH stimulation from experiments 1 and 2 were pooled and examined by way of a principal components analysis (reduces multicollinearity) and a multidimensional scaling analysis. Only data from the first week of stimulation in experiment 1 were included in the analysis to be consistent with the protocol of experiment 2 in which the rats received stimulation for just one week. Thus the

analysis was based on the results from 22 VMH and 23 extra-VMH animals.

### **Principal Components Analysis**

A principal components analysis (PCA) is a statistical technique that indicates which variables in a particular set of variables form coherent subsets that are relatively independent of one another (Tabachnick & Fidell, 1989). Those variables that are correlated with one another but largely independent of others are combined into factors (Tabachnick & Fidell, 1989). The variables considered in the present analysis were the three histological planes (anterior-posterior, medial-lateral, dorsal-ventral), weight gain, and food intake. The results of the analysis yielded four factors; weight gain and food intake were combined into one "mass" factor ( $r=.65$ ,  $N=45$ ) and the others were the three planes of electrode placement. These factors were then used in the multidimensional scaling analysis.

### **Multidimensional Scaling Analysis**

Multidimensional scaling, like PCA, can be categorized as a reduction procedure (Berenson et al., 1983). However, whereas PCA extracts a smaller set of factors from a larger set of variables, MDS uses a measure of similarity among objects to generate a spatial representation that consists of a geometric configuration of points, similar to a map, in

"n" dimensional space (Berenson et al., 1983; Kruskal & Wish, 1978). Essentially, this configuration reflects the hidden structure in the data. One of the most important ways to examine it is simply to look at the arrangement of points, where each point has been labelled to indicate which object it represents (Kruskal & Wish, 1978). It is the task of the researcher to assign theoretical meaning to this spatial representation.

Conventionally, MDS is often used in an exploratory way. Kruskal and Wish (1978) give the following example: Let's say that each respondent in a national sample evaluated 12 possible candidates for President of the United States. In this particular case multidimensional scaling helps to answer questions such as how similarly the public views the candidates, and what leads individual citizens to their decisions. The application of MDS to the data reduces the data about 12 candidates to "n" dimensions (in this example, partisanship and ideology) that represent the hidden structure of the data. By finding key differences between political candidates at opposite ends of each of these dimensions, it is possible to develop indicators of variables that can be measured in future elections (Kruskal & Wish, 1978).

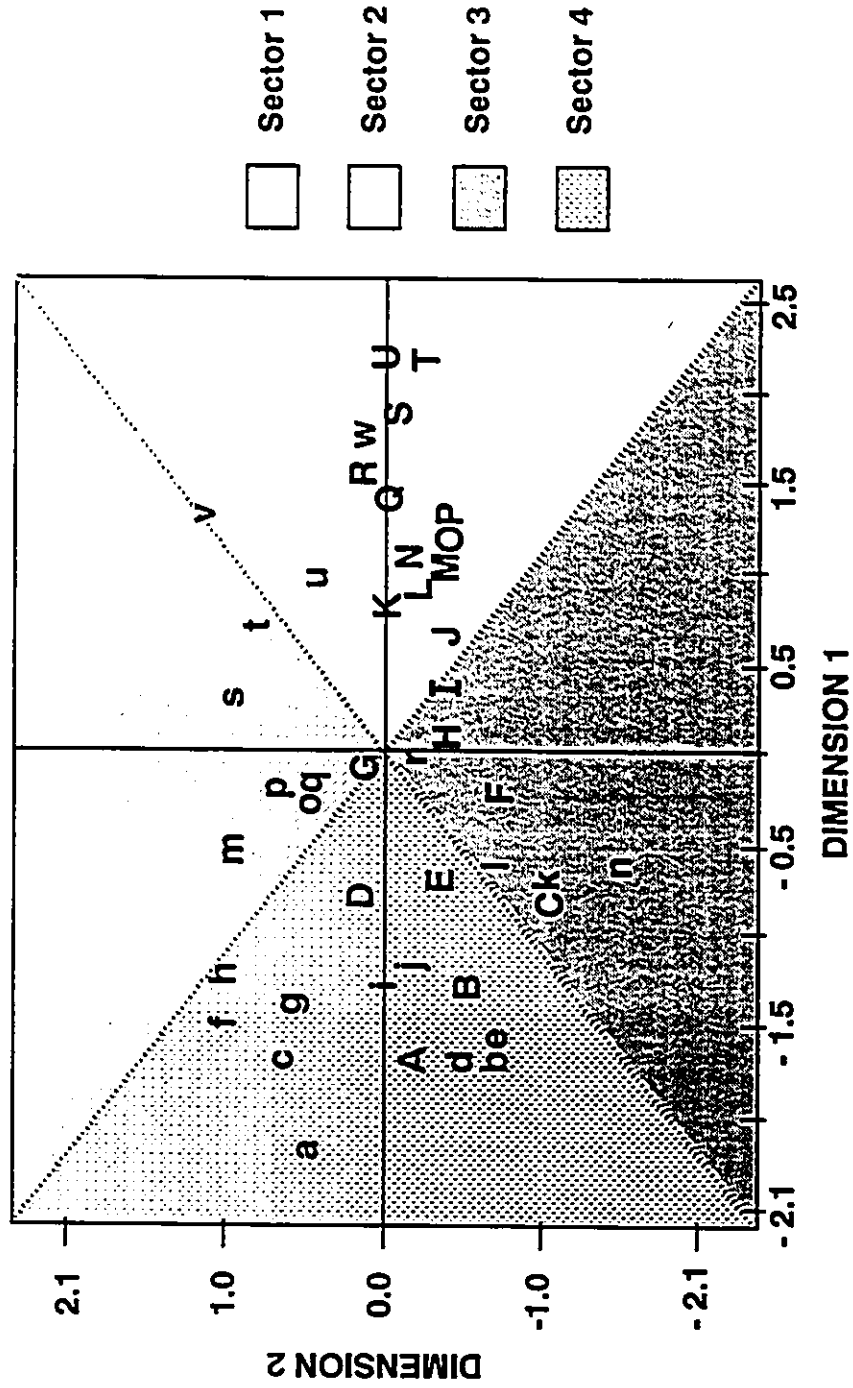
We have employed MDS in a different way by using it as a confirmatory, rather than exploratory, technique. The reason for this is strictly practical; two of the variables

investigated, weight gain and food intake (i.e. the mass factor) are experimental outcome measures and as such, represent months of work. In addition, the histological coordinates could not be verified until after the completion of the experiment. Another consideration is that MDS requires a substantial data set, which in this instance translates to data from a large number of rats; this was not available until the end of experiments 1 and 2. Therefore, in the present situation, the most appropriate time to conduct the analysis was after data collection was concluded. This being the case, the use of MDS was also attractive from another perspective; it provided a method for combining, and systematically examining the data from the two experiments. It was reasoned that the outcome of the MDS analysis should be consistent with the results from the more rigorous statistical analyses performed on the separate data sets. This assumption was borne out.

One of the first steps in the actual analysis involves the generation of a proximity matrix (a proximity is the number that indicates how similar two objects are). This was created for the 45 rats based on the histological data and the factorial scores from the principal components analysis. Metric scaling, which attempts to maintain a linear (or other functional) relationship between the plotted objects and actual distances (Berenson et al., 1983) was used to determine the MDS solution. The outcome of the

analysis yielded a two dimensional solution with an  $r^2$  of 0.94, and a Kruskal stress value of 0.12. The former is the squared correlation between the proximities and the distances between points (in the present case each point represents the data from one rat) mapped on a two dimensional coordinate system. Theoretically, the distances between points should correspond to the proximities (Kruskal & Wish, 1978); hence,  $r^2$  should equal unity. The stress value, on the other hand, essentially measures "badness-of-fit" (Diekhoff, 1992). As a rule, an  $r^2 \geq .90$  or a stress value of .15 or lower can be considered to provide an adequate fit between proximities and mapped distances (Diekhoff, 1992). Therefore, it was concluded that the fit between proximities and distances in the present case was satisfactory and the next step was to interpret the results. An inspection of the two dimensional multidimensional scaling solution for the proximity data (see Figure 14) suggests that the vertical dimension depicts the anteroposterior histological plane and the horizontal dimension represents the mass factor. Specifically, on the vertical axis, rats with anterior electrode placements have the highest positive values; these values decrease and eventually become negative for increasingly posterior placements. Similarly, the most negative values at the extreme left of the horizontal dimension are associated with the rats that showed the greatest weight gain (a component

Figure 14. The multidimensional scaling configuration obtained for data from 45 rats in Experiments 1 and 2 that received electrical stimulation trials to either the VMH or extra-VMH areas for a period of 1 week. The VMH group is represented by upper case letters and the extra-VMH group by lower case letters. The vertical dimension depicts the anteroposterior histological plane, and the horizontal dimension represents the mass factor. The dashed lines drawn on the MDS configuration divide the map into four sectors that are labelled 1, 2, 3, and 4. Sectors 2 and 4 show that rats with electrode placements within the VMH tend to gain less weight than those with placements at similar anteroposterior coordinates but outside of the VMH (extra-VMH group). Sector 3 shows that posterior electrode placements are associated with relatively high weight gains because individual points tend to cluster in the left portion of the quadrant. Sector 1 indicates that the most anterior electrode placements are associated with variable weight gain, as demonstrated by the scatter of points throughout this area.



of the mass factor); animals with the lowest weight gain are located at the extreme right of this dimension. Tables 5 and 6 show the data for each case depicted on the scatter diagram according to its order of appearance, from left to right, on the horizontal axis. Food intake, and the anterior/posterior (A/P), medial/lateral (M/L), and dorsal/ventral (D/V) histological coordinates are also shown. It is obvious, from these tables, that weight gain (and food intake) exhibit a decreasing trend. Recall that food intake and weight gain were combined into one mass factor because of their high positive correlation ( $r=.65$ ). However, the central issue of this thesis is whether there is a difference in weight gain between the VMH and extra-VMH groups. Therefore, to facilitate interpretation of the analysis, weight gain is considered instead of the combined mass factor. Note that food intake could just as easily have been chosen.

The dashed lines drawn on the MDS configuration (Figure 14) divide the map into four sectors. From our perspective, Sectors 2 and 4 are the most interesting because they contain the highest concentration of points. These points represent rats with electrode placements in the mid to anterior regions of the hypothalamic area. The examination of specific cases within these quadrants suggests that rats with electrode placements within the VMH nucleus (2.12 to 2.56 behind bregma) gained the least weight (sector 2) and

TABLE 5

## VMH Group

Case	Weight	Food	Coordinates		
	Gain (g)	Intake(g)	A/P	M/L	D/V
A	29	233	-2.30	0.4	9.2
B	27	225	-2.56	0.4	9.1
C	37	188	-3.14	0.5	9.3
D	32	200	-2.12	0.4	9.5
E	28	204	-2.56	0.8	9.7
F	28	188	-2.80	0.4	9.5
G	24	196	-2.12	0.7	9.3
H	20	195	-2.56	0.5	9.3
I	27	171	-2.56	1.0	9.7
J	18	183	-2.56	0.4	9.4
K	14	188	-2.30	0.9	9.2
L	15	179	-2.56	0.5	9.6
M	15	176	-2.56	0.8	9.4
N	10	187	-2.30	0.7	9.0
O	17	171	-2.56	0.3	9.2
P	11	180	-2.56	0.4	9.5
Q	13	170	-2.30	0.7	9.6
R	9	175	-2.12	0.3	9.1
S	16	149	-2.30	0.5	9.2
T	8	157	-2.56	0.5	9.5
U	7	159	-2.30	0.9	9.5
V*	0	154	-2.56	0.7	9.5

\* Case V lies beyond the right boundary of the scatter plot.

TABLE 6

## Extra-VMH Group

Case	Weight	Food	Coordinates		
	Gain (g)	Intake (g)	A/P	M/L	D/V
a	38	231	-1.80	1.0	9.5
b	30	231	-2.80	0.9	9.1
c	35	219	-1.80	0.5	10.0
d	30	231	-2.56	0.9	9.0
e	38	205	-2.56	1.5	9.9
f	40	204	-1.40	0.2	9.5
g	44	192	-1.80	1.0	10.0
h	28	224	-1.40	0.5	9.7
i	31	214	-2.12	0.2	9.0
j	38	199	-2.30	1.1	9.0
k	32	194	-2.80	0.3	8.6
l	26	205	-2.56	1.4	9.2
m	27	203	-1.80	0.1	8.5
n	24	207	-3.30	1.4	9.2
o	23	205	-1.80	0.3	9.4
p	36	177	-1.80	1.0	9.0
q	28	187	-2.30	0.7	10.2
r	24	195	-2.30	0.7	8.9
s	24	185	-1.40	1.2	9.5
t	16	190	-1.80	1.5	9.2
u	22	169	-1.80	1.0	9.2
v	12	181	-1.40	1.4	9.4
w	10	168	-2.12	0.9	9.4

rats with electrode placements at similar anteroposterior coordinates, but outside the VMH, have a greater weight gain (sector 4). This analysis is consistent with our previous work (Stenger et al., 1991; Bielajew et al., 1994) in that it suggests a difference in weight gain between VMH and extra-VMH groups. It should be emphasized that an MDS analysis is only suggestive, and this is why it is important to interpret it within the context of the results from Experiments 1 and 2. Nevertheless, it is interesting that there appears to be a recurrent theme that emerges no matter how the data are manipulated; stimulation of VMH and extra-VMH regions do not produce identical results. However, these differences may be due to dissimilarities in activity, food intake, or, in the case of the second experiment, an unexplained difference between groups that only emerged after surgery.

The other two sectors also provide some interesting insights into the data set. Specifically, Sector 3 shows that posterior electrode placements are generally associated with higher weight gain because the individual points tend to cluster leftward; this is true whether the rats had VMH or extra-VMH electrode placements. Such an outcome is consistent with the proposal that stimulation of anterior to mid VMH placements may inhibit weight more effectively than at more posterior sites (Bielajew, Stenger, & Schindler, 1994). Finally, Sector 1 shows that the most anterior

electrode placements are associated with variable weight gain, as demonstrated by the scatter of points throughout this area.

In the end, the most salient finding of this analysis is that extra-VMH and VMH groups can be distinguished on the basis of a combined mass factor (representing weight gain and food intake); this suggests that electrical stimulation of these regions produces differential effects. The strong positive correlation between food intake and weight gain, for the VMH group in particular ( $r=.67$ ,  $n=22$ ), indicates that the depression in weight gain is associated with a reduction in food intake. Therefore, the results of the MDS analysis suggest that it would be prudent to monitor the amount of food eaten by the rats, as well as their activity levels (Bielajew et al., 1994), in any subsequent work that examines the effects of chronic VMH stimulation. As a final point, using a MDS analysis to examine the present data is interesting in itself. This technique clarified the relationships between different variables (be they dependent or independent), and highlighted consistencies, not only within the data set, but with the other statistical analyses performed in Experiments 1 and 2 on subsets of the data. It has been suggested that the effects of electrical stimulation on VMH and extra-VMH regions are different (Stenger et al., 1991; Bielajew et al., 1994); the results of the MDS analysis support this view.

## EXPERIMENT 3

An increased lipolysis and/or decreased lipogenesis may contribute to the reduced weight gain observed in rats that receive chronic VMH stimulation (Stenger et al., 1991). Pawson (1988a) also suggests that overall body composition may be altered as a result of this type of stimulation. One way to examine this possibility is to determine the body composition of control and experimental rats several times throughout the experimental period; the comparison of the results of both groups of animals would clarify whether or not changes in fat and/or lean body mass was associated specifically with VMH stimulation. Unfortunately, direct carcass analysis, the method most commonly used to determine body composition in laboratory animals, can only be performed once on any given animal. A more attractive procedure for chronic stimulation studies is one that allows body composition to be tracked in the same rat over time. The measurement of total body electrical conductivity (TOBEC) provides a non-invasive assessment of body composition in the living animal and thus appears to be ideal for this type of work. Indeed, this technique has been widely used to estimate body composition in both animals and humans (Baer et al., 1993; Bellinger & Bernardis, 1991; Bracco, Yang, Segal, Hashim, & Van Itallie, 1993; Lukaski, 1987; Presta, Segal, Gutin, Harrison, & Van

Itallie, 1983; Presta, Wang, et al., 1983; Rumpler, Baer, Kressler, Howe, & Barnes, 1992; Segal, Gutin, Presta, Wang, & Van Itallie, 1985; Van Loan, Belko, Mayclin, & Barbieri, 1987; Van Loan & Mayclin, 1987; Van Loan, Segal, Bracco, & Mayclin, 1987). The basis for this measurement is the different conductive properties of lean body mass and fat mass; the former has a greater conductivity due to its higher electrolyte content (Pethig, 1979). The EM-SCAN model SA-2 small research animal body composition analyzer (EM-SCAN Inc., Springfield, IL) uses this measurement principle to estimate lean body mass and to indirectly predict body fat. It consists of a coil that generates an electromagnetic field within a measurement chamber; the TOBEC value is based on the difference between the coil's impedance when the chamber is empty and when it contains the animal (EM-Scan Model SA-2 Operation Manual, 1991). Changes in body composition can be tracked in the same animal because the measurement of TOBEC is noninvasive. Therefore, the use of this technology could provide insight into whether or not VMH stimulation alters body composition in any way. Hence, the third experiment was designed to evaluate how accurately the SA-2 small research animal body composition analyzer (EM-SCAN Inc.) predicts body composition in rats. The accuracy of fat mass predictions was the primary concern. There is some evidence to suggest that the TOBEC-derived measurements are less accurate for

total body fat than lean body mass (Baer et al., 1993; Lin, Reed, & Sun, 1992). Baer et al. (1993) state that small errors in estimating lean body mass might lead to large errors in calculating total body fat (which is predicted by subtracting lean body mass from total body weight) because body fat represents a relatively small percentage of overall body composition compared to lean body mass.

The equation used for the prediction of lean body mass, supplied by the manufacturer and developed for use with male Sprague-Dawley rats is:

$$\text{FFM} = 0.85184 \times L^{0.93812} \times E^{0.93812} - 5.84411$$

where FFM is fat-free mass, L is naso-anal distance, and E is the SA-2 reading. A similar equation has not been determined for the Long-Evans strain, which is the strain used in our previous work (Stenger et al., 1991; Bielajew et al., 1994) and the present study. However, because we were interested in examining the correlation between TOBEC values and fat pad weights (see below), the equation derived for Sprague-Dawley rats was felt to be adequate for our purposes.

It has been shown that an animal's retroperitoneal (also referred to as perirenal) and epididymal fat pad weights are highly correlated with overall body fat ( $r = 0.95$ ) as determined by carcass analysis (Himms-Hagen &

Cui, 1991). Therefore, in the present study the validity of the TOBEC measurement was assessed by comparing predicted body fat (in grams) with these two fat deposits (in grams). Previous work has also shown that factors such as animal position and core temperature loss contribute to increased variability in TOBEC estimates of body composition (Lin et al., 1992; Tobin & Finegood, 1991; Walsberg, 1988). Consequently, core temperature was monitored to ensure that it remained within an acceptable range, and the effects of position on TOBEC values were also evaluated.

## Method

### *Subjects*

Forty-six male Long Evans and two Sprague Dawley rats (Charles River, Quebec), weighing 210-505 g, were individually housed in plastic cages and given free access to laboratory chow and water. They were maintained on a 12 hr light and 12 hr dark cycle with light onset at either 0700 or 0900 hrs.

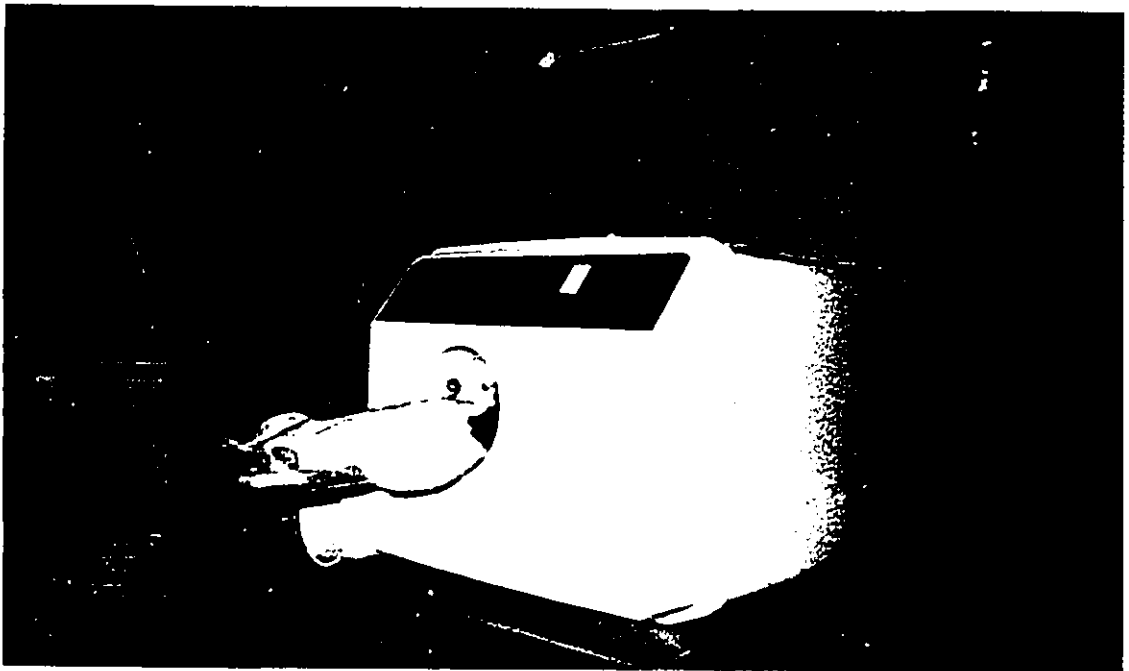
### *TOBEC Measurement*

All TOBEC readings were obtained during the light phase. To ensure that the SA-2 system was operating correctly, a measurement was made using the reference phantom supplied by the manufacturer (EM-SCAN Inc.) at the beginning of each measurement session.

Each rat was anaesthetized with a single injection of Innovar-vet (.2 mg/kg) combined with Midazolam (2.5 mg/kg) prior to insertion into the measurement chamber and both core temperature (measured with a rectal probe) and naso-anal distance were recorded. To minimize heat loss, the animals were placed on a heating pad while these measurements were taken. The rat was then placed in a supine position on the appropriately sized carrier and inserted slowly into the chamber (see photograph, bottom panel). All measurements were taken using the peak mode. The use of this mode dictates that the rat must be positioned forward on the animal carrier with the tip of its nose approximately 1 cm from the carrier's distal end. Also, the rat must enter the measurement chamber slowly; it took approximately 10 seconds to fully insert the subject into the chamber.

Each TOBEC value was based on 10 measurements. Two TOBEC values were obtained for younger rats - one with the tail extended in a loop so that the entire length of the measurement chamber was occupied and another with the tail tucked under the body of the animal (see photograph, top and middle panels). In the former case, the tip of the rats tail was secured to the rats right paw with a piece of masking tape (it had been previously established that the presence of the masking tape did not alter the TOBEC prediction). These two conditions were counterbalanced so

Figure 15. The top and middle panels show the two positions used for younger (smaller) rats during the measurements of total body electrical conductivity (TOBEC) by the SA-2 small research animal body composition analyzer. In the top panel the tail of the rat is extended in a loop so that the entire length of the measurement chamber is occupied; in the middle panel the tail is tucked under the body of the animal. The bottom panel shows the rat being inserted into the SA-2 model.



that an equal number of rats had their first trial in "tail extended" and "tail under" positions. Older animals could only be tested in the tail under position because their bodies occupied the full length of the measurement chamber.

On removal from the chamber, core body temperature was recorded and rats were euthanized with an overdose of sodium pentobarbital. The body cavity was then opened and the retroperitoneal and epididymal fat pads removed and weighed.

#### ***Exclusion Criterion***

A rat was excluded from the study if the coefficient of variation exceeded 3%, which is the accuracy specification for the SA-2, for any TOBEC value (EM-SCAN Inc. Model SA-2 Operation Manual, 1991). The coefficient of variation, a scalar term of the standard deviation divided by the mean, is expressed here as the differences between the TOBEC prediction and the reference method (e.g. carcass analysis).

### **Results**

The combination of the short acting anaesthetic (Innovar-vet) with the benzodiazepine (midazolam) produced a suitable level of anaesthesia and muscle relaxation for the determination of TOBEC values.

The mean core body temperature of the 46 rats in the study was  $37.2^{\circ}\text{C} \pm .4$  (SD) before insertion into the SA-2 measurement chamber and  $36.3^{\circ}\text{C} \pm .4$  (SD) when all

measurements were completed (each TOBEC value is based on 10 readings). The entire procedure (recording of naso-anal distance, core temperature, and collection of TOBEC values) took no more than 15 minutes.

Three rats were removed from the study because their respective coefficients of variation exceeded 3% of the TOBEC value (3.04%, 3.12%, 3.46%). A fourth rat was excluded because its predicted body fat, as determined by the TOBEC value, was negative. Therefore, the analysis was carried out on the remaining 42 rats - 40 Long Evans and two Sprague Dawley. The mean coefficient of variation for these animals was  $1.37 \pm .61$  (SD).

The results show that a positioning effect is observed with younger, (that is, smaller) rats. The reading was found to vary depending on the position of the tail within the measurement chamber. Specifically, curling the tail under the animal's body (the position necessary to use with mature rats) leaving a portion of the measurement chamber empty produced a different reading than positioning the tail so that the entire length of the measurement chamber was occupied. Fifteen rats ranging in weight from 215 to 339 g and 19-22 cm in length were assessed in these two positions. Their TOBEC values are shown in Table 7 along with the value for a larger animal for comparison (380 g, 23 cm). T-tests for correlated samples showed that the position of the tail within the measurement chamber affected the predicted

TABLE 7  
Lean Mass and Percentage Body Fat for Tail Under  
and Tail Extended Positions

RAT	WEIGHT	LENGTH (cm)	TAIL UNDER		TAIL EXT.		DIFF. %FAT
	(g)		LM(g)	%FAT	LM(g)	%FAT	
716	215	19.0	193.5	10.0	203.9	5.2	+4.8
719	216	19.5	200.3	7.3	215.8	0.1	+7.2
709	233	19.5	211.4	9.3	228.5	1.9	+7.4
720	251	20.0	231.1	7.9	241.0	4.0	+3.9
708	272	20.5	249.1	8.4	259.4	4.6	+3.8
728	282	20.5	266.4	5.5	270.2	4.2	+1.3
718	295	21.0	274.8	6.8	282.6	4.2	+2.6
724	298	21.5	277.5	6.9	276.9	7.1	-0.2
713	303	22.0	276.4	8.9	286.5	5.4	+3.4
711	311	21.0	275.6	11.4	291.8	6.2	+5.2
710	318	21.5	300.7	5.4	303.5	4.6	+0.8
712	318	21.75	300.0	5.7	306.7	3.6	+2.1
715	327	21.75	303.6	7.2	303.7	7.1	+0.1
722	335	21.5	305.6	8.8	316.8	5.4	+3.4
726	339	22.0	302.4	10.8	296.1	12.7	-1.9
714*	380	23.0	354.2	6.8	356.1	6.3	+0.5
x =	288	20.9	264.6	8.0	272.2	5.1	+2.9
SEM =	11	0.2	10.0	0.5	9.1	0.7	0.7

\*data not included in descriptive statistics.

percentage of body fat (the mean difference for the two positions was 2.9%,  $p < .01$ ). Likewise, estimates of lean body mass were significantly different for the two positions (the mean difference for the two positions was 7.7 g,  $p < .01$ ).

Two TOBEC values were obtained for each of seven mature rats; these results are shown in Table 8. These animals, due to their size, could only be measured with their tail wrapped under their bodies. The first TOBEC value was used in the calculation of the correlation coefficient, and the second was used to give an index of the reliability of the instrument. There was no statistically significant difference in either percentage body fat or lean body mass between the first and second TOBEC values for mature rats.

The best correlation ( $r = .83$ ) between combined epididymal and retroperitoneal fat pad weights, and TOBEC body fat prediction was obtained when smaller animals ( $n = 15$ ) had their tails extended so that the entire length of the measurement chamber was occupied. This correlation fell to .78 when the tail was wrapped under the rat's body. The scatterplot of the data is shown in Figure 16A.

In Figure 16B the data have been converted from grams to percentage body weight. This normalizes the values but truncates the range and results in a reduced correlation coefficient ( $r = .70$ ). All correlation coefficients were statistically significant ( $p < .01$ ,  $df = 41$ ).

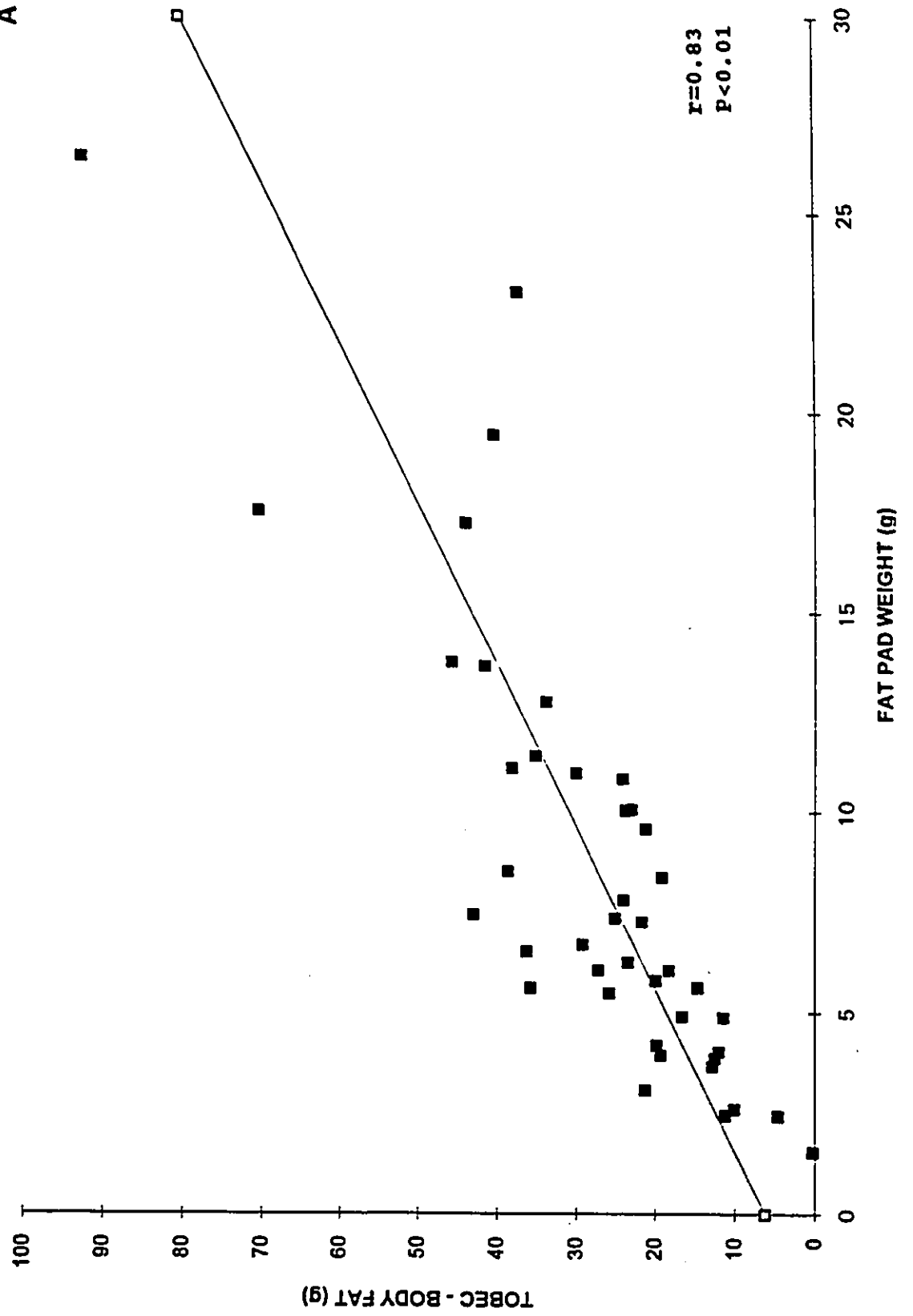
TABLE 8

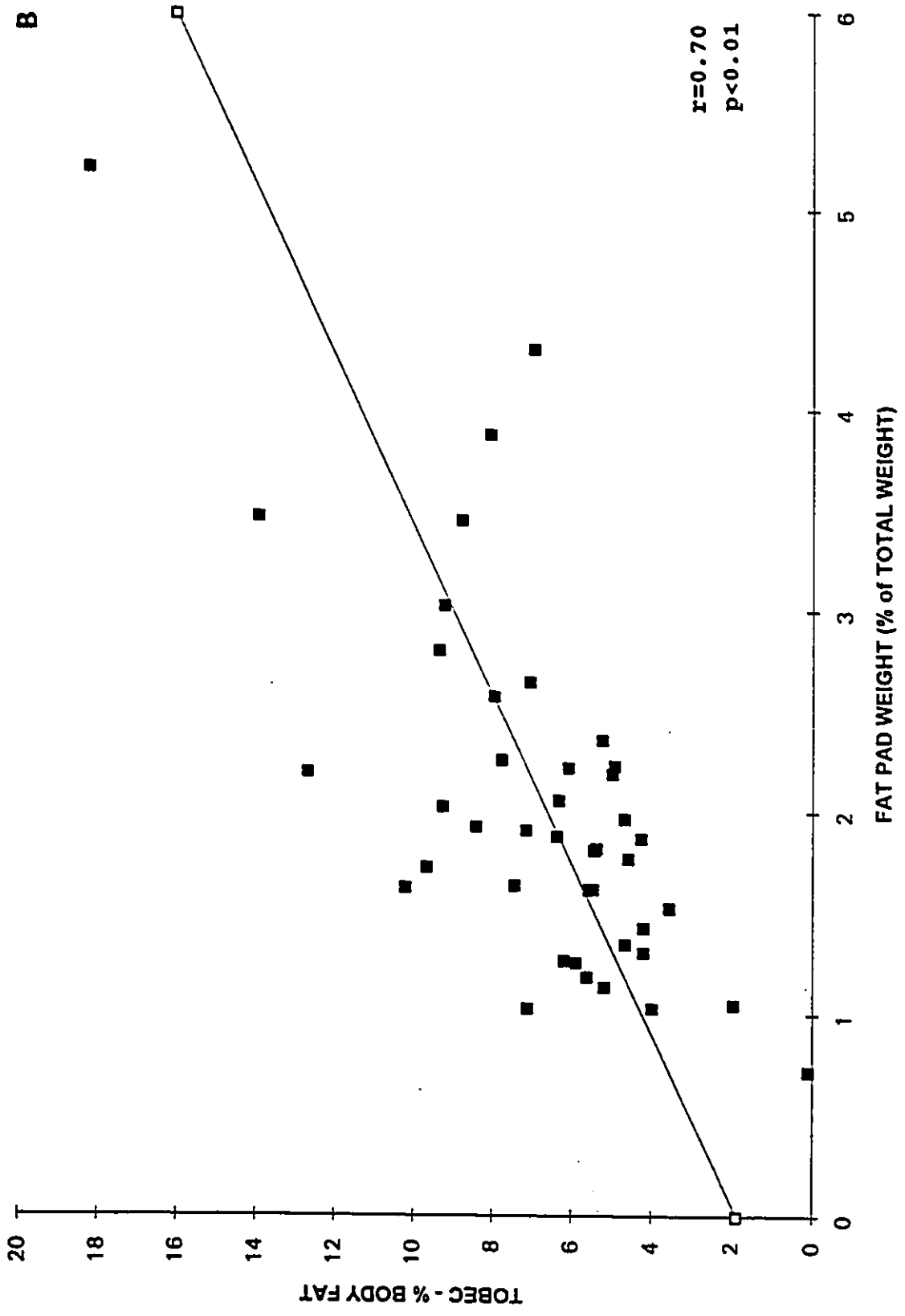
## Reliability of TOBEC Values

RAT	WEIGHT (g)	LENGTH (cm)	TOBEC 1		TOBEC 2	
			LM (g)	%FAT	LM (g)	%FAT
731	437	24.0	411.4	5.9	407.2	6.8
732	449	24.0	429.9	4.2	425.8	5.2
733	460	24.5	436.0	5.2	431.7	6.2
729	495	25.0	465.0	6.1	458.8	7.3
730	490	25.0	444.3	9.3	439.2	10.4
SD-A	505	25.75	434.8	13.9	437.0	13.5
SD-C	506	24.5	413.5	18.3	419.3	17.1

Figure 16A-B. The first graph (A) represents the scatterplot and correlation coefficient between TOBEC predicted body fat (g) and fat pad weight (g). The second graph (B) shows the scatterplot and correlation coefficient between TOBEC predicted body fat and fat pad weight when both are expressed as percentages of total body fat.

A





## Discussion

An Innovar-vet/Midazolam drug combination is an appropriate anaesthetic for this type of work for several reasons. First and foremost, Innovar-vet is a short-acting anaesthetic that has a rapid onset; thus, the drop in an animal's core temperature due to the effects of the drug is minimized. In addition, narcotic reversants such as naloxone and nalbuphine are very effective in reversing the anaesthesia, thereby reducing the time that the rat is unconscious even further. Other desirable characteristics are that the drug combination of Inovar-vet and midazolam can be administered in a single intraperitoneal injection and preliminary work shows that animals appear to tolerate repeated injections well (see Flecknell, 1987).

The rat's position within the measurement chamber is critical to the accuracy of the measurements; it must be in a completely flaccid state when they are taken. Therefore, midazolam (because of its muscle relaxant properties) was added to the Innovar Vet. This combination produced a suitable level of anaesthesia and muscle relaxation for SA-2 measurements.

The accuracy criterion specified by the manufacturer is a coefficient of variation of  $\leq 3\%$  for the SA-2 model. For this reason rats with a variability greater than this value were excluded from the study. It is not clear why this

occurred in three animals. According to the SA-2 operating manual (1991), both the subject's geometry (i.e. position within the measurement chamber) and temperature are critical to the accuracy of the measurements. The level of anaesthesia and muscle relaxation for these rats were similar to that observed in the remaining 42 animals. Therefore, it seems unlikely that the position of the rat in the chamber was responsible for the increased variability in the TOBEC value. Also, changes in core temperature, which were monitored by taking the rat's rectal temperature before insertion into the chamber and after the completion of the measurement process, remained within the acceptable range of  $\pm 2^{\circ}\text{C}$  as defined in the SA-2 operating manual. The average daytime core temperature of the rat is known to range from  $36.6\text{--}37.9^{\circ}\text{C}$  (Gordon, 1987, 1993; Herrington, 1940); the temperature range from the start to the end of the measurement process for the three rats that were removed from the study was  $35.3\text{--}37.7^{\circ}\text{C}$ .

Curiously, a negative value for percentage body fat was obtained in one rat. The animal in question weighed 236 grams, had a naso-anal distance of 20.25 cm, combined epididymal and retroperitoneal fat pad weights of 2.4 grams, and in no way appeared different from the other animals. The negative values were obtained when the rat was in the tail-extended position. However, in this same rat, a positive TOBEC value (2%) was observed when the tail was

wrapped under the its body. These results are consistent with those obtained overall in that the predicted value for percentage body fat tends to be larger when the tail is curled under the rat than when it is extended so that the entire length of the measurement chamber is occupied (Table 7).

The finding of a negative percentage body fat has been reported elsewhere. Baer et al. (1993) developed a prediction equation for lean body mass based on carcass analysis that they claimed was more accurate than the one supplied by EM-SCAN Inc. However, when the new equation was used, they observed fat predictions that were less than zero in some rats. They suggested that this outcome was the result of indirectly estimating body fat (the difference between lean body mass and total body weight) and that small errors in predicting lean body mass may translate to large errors in estimating percentage body fat. However, there is a caveat to these findings; Baer et al. (1993) placed the tail of the rat outside the measurement chamber, a position that is not recommended by the manufacturer.

In the present study, wrapping the tail under the rat's body appears to produce slightly inflated TOBEC values for percentage fat mass in young animals (refer to Table 7). When the tail was extended in a loop so that the entire length of the measurement chamber was occupied, the TOBEC value was significantly less for percentage fat mass.

This is consistent with the work of Tobin and Finegood (1991) who also found that TOBEC predictions were affected by tail position. Furthermore, for the results reported here, the best correlation between fat pad weight and total body fat was obtained for the 42 rats when data for a tail extended position were used for the young animals (n=15). As a rat becomes larger (longer), the tail-under position more closely approximates the tail-extended position, and this seems to be reflected in a greater similarity in the TOBEC values (see Table 7). In large rats only one position is possible; the tail must be wrapped under the animal for the rat to fit into the measurement chamber. Table 8 shows that the reliability between TOBEC values for the same rat is good, at least with respect to repeated measurements in large rats. No significant difference was observed in either lean body mass or percentage body fat between the first and second TOBEC values.

Lin, Reed, and Sun (1992) reported an  $r$  value of 0.90 for the regression of percentage whole body fat on TOBEC predicted fat. The findings of the present study show a weaker relationship between fat pad weight and the TOBEC prediction ( $r=0.83$ ). Two factors may contribute to the slightly reduced correlation in the present study. Specifically, body fat was estimated by fat pad weight rather than total carcass analysis, although the two are highly correlated ( $r=0.95$ ) and the equation used to predict

the TOBEC value was developed for Sprague-Dawley rather than Long-Evans rats. As mentioned earlier, because we were interested in examining the correlation between TOBEC values and fat pad weights, the use of this equation was deemed adequate for our purposes; any error introduced by the equation should be constant and therefore have minimum impact on the outcome measure (i.e. the correlation coefficient).

There are a number of conclusions that can be drawn from the present study. An Innovar Vet/midazolam combination is a very effective anaesthetic for this type of work. Innovar-vet is a short acting anaesthetic that has a rapid onset. These are desirable characteristics because the whole TOBEC measurement procedure takes less than 15 minutes; the rapid onset and short action ensures that heat loss during this time is minimized. In addition, this drug combination left the rats in a completely flaccid state, which is the degree of muscle relaxation necessary for accurate TOBEC estimates. This ensured that the geometry of the rat within the measurement chamber would meet the specifications outlined by the SA-2 manufacturer, and allowed the evaluation of the effect of minor changes in position. The results show that the position of rat in the measurement chamber is crucial, especially when the peak mode is used in young animals. Even small changes in position, such as altering the location of the tail within

the measurement chamber, significantly affect the TOBEC value. Hence, this indicates that different positions for young and old animals may be required for accurate TOBEC predictions. Finally, the reliability of the readings appears to be reasonable with mature rats (based on repeated TOBEC predictions in the same animal).

The outcome of a preliminary study is also of interest when considering the question of the validity of the SA-2. This work suggests that serial estimations of body composition may be inaccurate. Specifically, TOBEC values were recorded at approximately 1-week intervals for 3 weeks in seven rats. The range of weights for the first set of readings was 349-417 grams; by the third set the range was 402-502 grams. All rats showed a steady weight gain throughout the experimental period, which was not reflected in the TOBEC predictions. Only two of the seven animals showed a progressive increase in fat mass over the three measurement sessions. It is possible that estimates of lean body mass are more accurate because this value increased in six rats over the experimental period, although the magnitude of increase did not always reflect the gain in weight. Novak et al. (1991) also found that there were problems associated with serial determinations of body composition in rats. They conclude that although correlations between TOBEC measurements and carcass analysis may be good when one TOBEC value is obtained, tracking

changes in body composition by way of serial TOBEC estimates may be problematic.

The results, when considered in their entirety, suggest that at this time the SA-2 small research animal body composition analyzer is not a valid and reliable measurement method for tracking changes in body composition in the same animal. However, it is hoped that changes to this technology will ultimately resolve the problems encountered both in the present study and by other researchers. A measurement method that allows the monitoring of changes in body composition within the same animal would truly be a great asset to any type of metabolic research.

## GENERAL DISCUSSION

The results of the first two experiments, along with the outcome of the multidimensional scaling analysis, strongly suggest that chronic electrical stimulation of the VMH inhibits weight gain in rats. In the case of the first experiment, there are two findings in particular that stand out. First, it seems clear that it is critical to include a stimulated control group given that approximately 50% of the difference in weight gain between the VMH stimulated and unstimulated groups could be accounted for by the non-specific effects of brain stimulation. However, the fact that there was only a significant difference in weight gain between the VMH implanted control and the VMH stimulated groups suggests that the inhibition of weight gain is related to VMH stimulation specifically; this represents the second important finding.

The purpose of the second experiment was to take a closer look at what factors may contribute to the reduction in weight gain following VMH stimulation. The results of this study confirmed the findings of the first experiment in that no significant difference in weight gain was observed between the two stimulated groups (VMH and extra-VMH). This is not surprising if one considers the magnitude of the effect. For example, the VMH may selectively inhibit weight gain by increasing lipolysis or decreasing lipogenesis.

Body fat represents a relatively small proportion of overall body mass; therefore, small differences in body weight may translate to large differences in body composition - specifically body fat. A screening procedure, such as the one used by Pawson (1988b), may be necessary to maximize this effect of VMH stimulation. Recall that he selected only those rats which showed the greatest stimulation induced increases in metabolic rate to be evaluated in his chronic study.

The time of light onset marks a transitional point in the diurnal cycle of the rat; metabolic rate shows a sharp decline, and there is a shift from a lipogenic to a lipolytic metabolism (Le Magnen & Devos, 1970). It is interesting that Atrens et al. (1985) found that VMH stimulation was associated with an increase in metabolic rate but was not correlated with a shift in respiratory quotient (a measure that indicates the type of fuel utilized by the animal). Specifically, their data showed that while medial hypothalamic stimulation leads to an increase in respiratory quotient (RQ), indicating a rapid stimulation-induced increase in glucose utilization, this effect is relatively short lived and returns to baseline by the time metabolic rate has reached its peak (about 15 minutes post-stimulation). This suggests that fats are the major fuel source (as is the norm for fasting rats) by the time metabolic rate is at a maximum. These results have some

interesting implications for chronic stimulation studies; for example, our rats received one minute of stimulation every nine minutes, whereas the inter-stimulation interval that Pawson (1988a) used was longer. The time interval between each minute of stimulation may, in fact, be very important because the longer interval may permit a shift to a lipolytic metabolism. In addition, Le Magnen and Devos (1970) found that a low level of lipogenesis was still observed in normal rats for the first hour after light onset. The authors found that over a 24 hour period, 77% of the fat synthesized during the night was remobilized by the rats during the following light period. These results, when considered together, suggest that VMH stimulation commencing at light onset could prevent the normal decrease in metabolic rate and eliminate any further lipogenic activity. Moreover, the interval between each period of stimulation may be important with respect to the fuel source being utilized; longer time periods may permit the mobilization of fats.

The results of the second experiment highlight the important contribution of the effects of locomotor activity to stimulation induced reductions in weight gain. There is a growing body of evidence suggesting that VMH stimulation promotes a non-insulin dependent uptake of glucose that is similar to that observed with exercise (Shimazu et al., 1991; Takahashi et al., 1992). Narita et al. (1993) believe

that the VMH may play a role in the integration of motor activity and energy metabolism. When considered together, these studies seem to suggest that increased locomotor activity may itself be an important consequence of VMH stimulation, and therefore should be at least weighed when evaluating any VMH stimulation effect.

In the end, one is left with the impression that, although the effect is not large, chronic stimulation of the VMH does lead to a reduced weight gain in rats. It bears repeating that the size of the effect in no way implies that it is insignificant; a relatively small reduction in weight gain may represent a substantial decrease in body fat. The main challenge in this work lies in identifying the factors that contribute to the reduction in weight gain. An increase in locomotor activity and metabolic rate, and an inhibition of food intake probably all contribute to this outcome. Furthermore, the characteristics of the animal, such as strain, sex, and age may influence the degree to which weight gain is depressed. Therefore, like the proverbial onion, there are many layers to the study of the effects of chronic VMH stimulation. This is not unexpected considering the complexity of the hypothalamus; it mediates many of the behaviours which are essential to the survival of the organism and the continuation of the species.

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