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MULTIPLE CONTROLS OF HYPERPHAGIA

by

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A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

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Abstract

MULTIPLE CONTROLS OF HYPERPHAGIA

by

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During the dynamic phase following ventromedial hypothalamic (VMH) lesions three major changes in the meal pattern of the rat have been observed. First, meal size increases without compensatory decreases in meal frequency. Second, under certain conditions both size and frequency of meals are elevated. Third, there is an increased amount of eating during the normally inactive phase of the daily activity cycle which usually occurs under conditions of illumination. In addition, characteristic changes in the meal pattern occur over two months following the lesion.

These alterations have suggested at least four possible mechanisms for the hyperphagia:

1) Decreased post-prandial satiety. Hyperphagia results from a defect in the mechanism controlling meal termination (satiety) without damage to the mechanism controlling meal initiation (hunger).
2) Decreased intermeal suppression of feeding. The animal becomes less sensitive to the events which maintain satiety between meals. The hyperphagia results from the animal feeding sooner than would normally be expected after meals of a given size.
3) Resetting body weight set point. Regulation of body weight is"normal" about an obese baseline. The degree of obesity which inhibits

excess feeding is set at a higher level in the VMH-lesioned rat.

4) Increased light phase intake. Increased intake results from a disturbance in the daily cycle of lipolysis and lipogenesis. The normal periodicity of feeding is lost and the rat eats in the daytime the same amount as in the night. Overeating is primarily the result of an increase in feeding during the light phase without a compensatory decrease in feeding during the dark phase.

The present investigation provides new data for the evaluation of these accounts of hyperphagia. First, the experiments of Becker and Kissileff (Inhibitory controls of feeding by the ventromedial hypothalamus. American Journal of Physiology, 1974, 226, 383-396.) were replicated with six female rats using a solid diet (Noyes pellets). This replication established that the Becker and Kissileff findings were not restricted to a particular diet although other studies using a solid diet are not entirely in agreement with the present. In the next two experiments, meal patterns in animals exhbiting increased intake without VMH lesions were examined to determine if the increased food intake was expressed in the same manner.

In experiment 2 employing four genetically obese Zucker rats and four nonobese siblings as controls, it was found that the genetically obese rats show an increased amount of feeding during the normally inactive (light) phase suggesting the possibility of a generalized loss of nychthemeral periodicity of feeding in hyperphagia. To explore this possibility further,

patterns of food intake were studied in seven lactating rats (experiment 3). In lactating rats, however, with the exception of only one period of time in the rats fed Noyes pellets, the percent of the total food intake consumed during the light phase remained the same as before parturition suggesting that hyperphagia and the nychthemeral periodicity of feeding are under independent neurological control. This possibility was further supported by experiment 4 employing 37 rats in which the strategy was to prevent the nychthemeral periodicity of feeding from influencing food intake by restricting animals to 12 hours of feeding. In this experiment VMH-lesioned rats in the immediate post lesion dynamic phase overeate and gained excessive amounts of body weight when restricted to 12 hours of access to food. Restriction to 12 hours of access to food, however, reduced intake and retarded rate of body weight gain in the lesioned animals after they had been allowed to become obese (30-65 days post lesion).

The present results eliminate increased light phase intake without compensatory decrease in dark phase intake as the sole reason for increased intake with VMH lesions. In addition, the failure of obese VMH-lesioned rats to exhibit "normal" feeding behavior in experiments 1 and 4 does not support the account of hyperphagia as the result of a resetting of body weight set point. From the present work it appears that the mechanisms of hyperphagia for VMH lesions include decreased intraprandial satiety, and decreased intermeal suppression of feeding possibly resulting from the uncoupling of the controls of eating from their metabloic consequences.

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Introduction

Over 30 years ago experimental obesity was produced in the rat by lesions in the area of the ventromedial hypothalamus (VMH)(Hetherington & Ranson, 1940), and it was soon determined that the cause of this obesity was overeating (Brobeck, Tepperman,& Long, 1943). These latter investigators showed that there were two phases of the weight gain caused by the overeating, a dynamic phase characterized by rapid weight gain and approximate doubling of food intake, and a static phase during which body weight rose slowly and asymptotically to a new plateau while food intake hovered at slightly higher than normal levels. Subsequent studies have detailed many of the alterations in feeding behavior which accompany the hyperphagia but have failed to provide a comprehensive explanation for the overeating. The first part of this introduction reviews the various explanations for the effects of VMH lesions from the dual-center theory to very recent neurochemical explanations. The second part of this introduction is a review of the various techniques used to study feeding behavior following VMH lesions and a discussion of the techniques and strategy used in the present investigation.

Dual-Center Theory

Based on the finding that lateral hypothalamic (LH) lesions produce aphagia and that combined LH and VMH lesions have the same effect as lateral hypothalamic lesions alone, Anand and Brobeck (1951) proposed that a feeding center localized in the LH received inhibitory fibers from the VMH, and that VMH destruction removed this inhibition and therefore led to overeating. This proposal was expanded into a dual-center theory of food intake which has been most succinctly summarized by Anand (1967) as follows:

> Experimental studies have provided evidence that there are two opposing mechanisms in the hypothalamus that regulate food intake (Anand, 1961; Anand & Brobeck, 1951; Anand, Dua & Shoenberg, 1955) namely, a mechanism in the lateral region of the hypothalamus which initiates feeding and is therefore designated the "feeding center," and one in the medial part of the hypothalamus which brings about satiety after a meal has been taken and is thus termed the "satiety center" (Brobeck, 1960; DeGroot, 1967). Feeding behavior is probably based on feeding reflexes operating through the spinal cord and brain stem levels, which are put into effect by sensory stimuli that make the animal aware of the presence of food (Brobeck, 1960). These reflexes are facilitated by the feeding center and inhibited from the satiety center, and the activities of these hypothalamic regions provide the basis for hunger and satiety states....The adjustment of food intake to energy expenditure is probably a hypothalamic function. As a result of feeding, certain changes are produced in the body which directly or indirectly stimulate the activity of the hypothalamic satiety center (and possibly also the higher cerebral regions). The satiety center by suppressing the activity of the feeding center, brings about the satiety state. Subsequently when the food eaten is disposed of, through conversion to heat, work or some form of stored energy, the activation of the satiety center is removed and the feeding center becomes active; this leads again to the state of hunger (Anand, 1962; Anand, 1963; Brobeck, 1955). (pp. 249-250)

Evidence for the Dual-Center Theory

In addition to the lesion experiments mentioned above, this conceptual scheme is supported by electrical and chemical stimulation experiments as well as electrophysiological and anatomical studies. Stimulation of the LH increases food intake (Delgado & Anand, 1953; Morgane, 1961; Smith, 1961; Wyrwicka & Dobrzecka, 1960) and has been used as a method of making animals obese (Steinbaum & Miller, 1964). Conversely, electrical stimulation of the VMH results in the inhibition of feeding (Olds, Allen, & Bries, 1971; Smith, 1961) or the termination of feeding (Wyrwicka & Dobrzecka, 1960). Chemical injection of hypertonic saline into the LH elicits eating while injection of the same substance into the VMH suppresses spontaneous feeding in hungry rats. The application of procaine to the LH suppresses feeding and its application to the VMH elicits eating in satiated animals (Epstein, 1960). Evidence of reciprocal activity between

the medial and the lateral hypothalamus has been provided by the electrophysiological work of Oomura and co-workers (1967). Anatomical connections between the VMH and LH have been reported by Arees and Mayer (1967). Hyperphagia and obesity have been produced in several laboratories by knife-cut transections between the VMH and LH (Albert & Storlien, 1969; Gold, 1970a; Sclafani & Grossman, 1969). However, none of these studies demonstrates the direction of information flow.

Evidence that the Dual-Center Theory is an Oversimplification

The conception that food intake is normally controlled by a "feeding center" in the LH and a "satiety center" in the VMH and that destruction of the VMH results in the loss of inhibition of the VMH upon the LH causing hyperphagia is an incomplete explanation of hypothalamic function in light of more recent anatomical and behavioral data. Apparently not all inhibitory fibers originating from the VMH responsible for the hyperphagic syndrome connect directly laterally with the LH since hyperphagia and obesity have been produced following coronal plane cuts anterior or posterior to the VMH (Albert, Storlein, Albert, & Mah, 1971; Grossman, 1971; Palka, Liebelt, & Critchlow, 1971). Moreover, Gold (1970a, 1970b) and Sclafani (1971) have reported that parasagittal cuts which are just limited to the lateral border of the ventromedial nucleus do not produce hyperphagia and obesity and only those cuts rostrolateral to the anterior tip of the ventromedial nucleus cause the overeating and obesity. These findings suggest that the fibers responsible for the hyperphagia syndrome are either part of a multisynaptic projection from the VMH; project to, rather than from, the VMH; or do not arise from or terminate on VMH cells (Gold, 1970a). In addition, it has recently been reported that inhibition of feeding in hungry rats caused by electrical stimulation of the VMH is

not altered by either knife cuts between the LH and VMH or LH lesions (Sclafani & Maul, 1974).

There is evidence that questions the notion that damage to the ventromedial nucleus (VMN) itself contributes to obesity. Hetherington and Ranson (1940) found that some of their animals which became obese had an intact VMN and that the lesion was located posterior to the VMN. Gold (1973) has recently reported that lesions restricted to the VMN do not result in overeating and obesity. VMN lesions only cause obesity when they overflow the VMN, and the magnitude of the obesity is proportional to the amount of overflow. According to Gold, the most effective area for obesity is the area immediately rostral to the rostral tip of the VMN where a group of noradrenergic fibers thought to derive from the ventral ascending noradrenergic bundle (Fuxe, 1965; Ungerstedt, 1971) crosses the midline within the suprachiasmatic decussation.

Recent Neurochemical Studies

Destruction of the noradrenergic pathway with electrolytic lesions at the midbrain level or the injection of 6-hydroxydopamine (6-HDA) into the ventral noradrenergic bundle at the same level as the oculomoter nucleus causes hyperphagia and obesity (Ahlskog & Hoebel, 1973). Evidence of persistent depletion of tel-diencephalic norepinephrine following VMH lesions and significant inverse correlations between tel-diencephalic norepinephrine and weight gains following VMH lesions supports the idea that damage to a noradrenergic bundle is at least partially responsible for the hyperphagia (Glick, Greenstein,& Waters, 1973). Moreover, it is becoming apparent that at least some of the impairments in feeding behavior which have been observed following hypothalamic lesions may be the result of damage to catecholaminergic neuronal pathways. 6-HDA administered

intracerebrally along the nigrostriatal bundle (Marshall & Teitelbaum, 1973; Ungerstedt, 1971), within the lateral hypothalamus (Marshall & Teitelbaum, 1973; Smith, Strohmayer,& Reis, 1972) or by way of the lateral ventricles (Zigmond & Stricker, 1972) causes impairments in feeding and drinking behavior which resemble the effects of LH lesions. It has been suggested that these effects are the result of the depletion of striatal dopamine which has been observed (Oltmans & Harvey, 1972; Ungerstedt, 1971; Zigmond & Stricker, 1972) to accompany the deficits in feeding behavior. Thus, in summary, effects somewhat similar to those observed following VMH lesions occur following damage to the ventral noradrenergic bundle and effects similar to LH lesions can be produced by damage to the nigrostriatal bundle, and these effects apparently are the result of depletions of catecholamines.

Experiments on "Hunger" Motivation

Another means of investigating the role of the hypothalamus in feeding is by assessing the behavior of lesioned rats on various motivational tests thought to be measures of "hunger." The conception of the VMH as a "satiety center" was supported by the experiments of Miller, Bailey, and Stevenson (1950) which demonstrated a paradox in the behavior of VMH-lesioned rats. These animals consumed more food than normal controls, but did not perform as well as normals on various motivational tests such as running down an alley, bar pressing on fixed-interval schedules, or consuming a quinine adulterated diet. Since the VMH-lesioned rats did not display increased hunger on these tasks, it was thought that the reason they eat more food is because once they started eating they were unable to stop as soon as normals. This explanation is incomplete because it is now apparent that the VMH-lesioned rat, at least

under certain circumstances, is as hungry or hungrier than normal rats and will perform as well or better on various behavioral tests (Beatty, 1973; Jaffe, 1973; Kent & Peters, 1973; Peters, Sensenig, & Reich, 1973; Sclafani & Kluge, 1974; Singh, 1973). Nevertheless, these experiments are still consistent with the idea the VMH-lesioned rat overeats because it lacks a normal ability to terminate ongoing feeding. A possible explanation for the findings that VMH-lesioned rats perform better than normal rats on various behavioral tasks is that the lesioned animals take longer to become completely satiated.

Meal Pattern Studies

Although the experiments cited above support the notion of a lateral hypothalamic and dopaminergic involvement in the initiation of feeding and ventromedial hypothalamic and noradrenergic involvement in its termination, they have neglected a direct examination of the pattern of initiation and termination of eating which is supposedly affected by hypothalamic neural activity.

Richter (1927) showed that the total daily food intake of the rat is the result of discrete short periods (5-10 minutes) of rapid and continuous eating separated by longer periods (2-4 hours) when the animal engages in non-feeding behavior such as sleeping or locomotion. Brobeck (1955) pointed out that the total daily food intake is the product of the mean meal size and meal frequency in analogy with other physiological systems such as cardiac output and minute volume of air intake. In addition, it has more recently been shown originally by LeMagnen and Tallon (1966) and reproduced by some (Balagura & Coscina, 1968; Snowdon, 1969; Thomas & Mayer, 1968), but not all workers (Collier, Hirsch, & Hamlin, 1972; Levitsky & Collier, 1968; Premack, 1965) that there is a positive and significant correlation

between meal size and the interval which follows it.

Although we do not know for certain at the present time whether meal patterns and food intake are independently controlled or whether food intake is controlled by modulation of meal-taking, it is reasonable that close examination of the effects of lesions on meal-taking patterns should aid in revealing the behavioral mechanism by which intake is modified by brain lesions. Analysis of brain lesions in conjunction with manipulation of meal patterns may answer the question of the relationship between total daily intake and the temporal pattern of intake.

Effects of VMH Lesions on Meal-Taking Patterns. Ventromedial hypothalamic lesions during the dynamic phase result in three major changes in meal-taking pattern of the rat. First, they result in increases in meal size without compensatory decrease in meal frequency (Balagura & Devenport, 1970; Brooks et al., 1946; Teitelbaum & Campbell, 1958; Thomas & Mayer, 1968). Another way of expressing this phenomenon is that the satiety ratio (size of a meal divided by the interval of time which follows it) is shortened (Becker & Kissileff, 1974; Panksepp, 1973). Second, under certain conditions (e.g., animals eating solid diets and several days after lesions have been placed in animals eating liquid diets) both the sizes and frequency of meals are elevated (Becker & Kissileff, 1974; Teitelbaum & Campbell, 1958). Third, there is an increased amount of eating during the normally inactive phase of the daily activity cycle which usually occurs when the lights are on (Becker & Kissileff, 1974; Balagura & Devenport, 1970; Kakolewski, Deaux, Christenson, & Case, 1971; LeMagnen, Devos, Gaudilliere, Louis-Sylvestre, & Tallon, 1973). In addition there are a series of progressive changes in the meal-taking pattern which occur following the lesion. Briefly these changes are: after an initial increase

in meal size and reduction in the period of satiety following meals of a given size, a reduction in meal size and increase in meal frequency; and then as the animals gain weight, a more gradual reduction in meal frequency while meal size remains at or slightly above its pre-lesion value. During this entire time the normal pattern of predominantly nocturnal feeding is replaced by equal feeding during both dark and light phases (Becker & Kissileff, 1974).

Mechanisms of Hyperphagia Suggested by Behavioral Results. The alterations in feeding behavior described above could be involved separately or in combination in the occurrence of hyperphagia. It is possible that some of the alterations may be crucial for hyperphagia while others may be merely side effects of the lesion which could be duplicated without hyperphagia by small discrete lesions within the area where large lesions produce hyperphagia. It is also possible that the alterations in meal-taking produced by the lesions reflect underlying neural events which result in hyperphagia. Finally, it is possible that the mechanisms controlling the pattern of eating may be completely independent of the mechanisms controlling hyperphagia. Let us consider therefore some of the mechanisms through which each of the above expressions of hyperphagia could be producing hyperphagia and the evidence favoring and refuting each.

Large meals without increase in meal frequency could result from a defective satiety system with a normal meal initiation system. This implies that the signals causing initiation and termination of each meal are independently controlled and that the effects of one meal do not carry over to the next. It seems likely that some signal causing initiation of eating (e.g., low blood glucose, empty stomach) are not simply opposite conditions from those causing its termination (e.g., low blood glucose can result in

initiating of eating but its converse does not immediately terminate a meal (Baile, Zinn, & Mayer, 1971; Smith, 1966; Yin & Tsai, 1973). However, the positive correlation of meal sizes with the intervals they follow (LeMagnen & Tallon, 1966; others) indicates that some of the meals may influence the onset of subsequent meals and this linkage therefore precludes the sole operation of the mechanism above because, as Teitelbaum (1967) has stated, "the hyperphagic would merely decrease the number of its meals to compensate for their increased size" (p. 322). Decreasing meal size by surgically removing a portion of the stomach does not diminish hyperphagia; the animal merely compensates by increasing its meal frequency (Brooks et al., 1946). In addition, Thomas (1971) has shown that when meal size is restricted by limiting food access for 20 minutes after each 4 ml of diet consumed, hypothalamic hyperphagic animals continue to overeat. While this evidence appears conclusive on the surface to preclude increased meal size as an essential ingredient for hyperphagia, it is open to criticism. Brooks et al., (1946) did not show that their hyperphagic rats ate more frequent meals than controls similarly gastrectomized and thus there is no evidence that hyperphagia can occur in VMH lesioned rats without enlarged meals. Thomas' animals were deprived for a period of time which just borders on the critical limit of what appears to be the normal length of some meals in the VMH-lesioned rat (see discussion of the criteria for defining intermeal interval in experiment 1), and it is therefore conceivable that meal size was not actually reduced by the procedure but that meals were instead prolonged. While numerous attempts have been made to test this hypothesis by use of pre-loads, all of them suffer from one or more deficiencies. Smith, Salisbury, and Weinberg (1961) used a forced-

feeding and food restriction schedule (1 hr/day) which was possibly aversive to the animals. Panksepp (1971) did not examine the effects of his infusion on single meals. Thomas and Mayer (1968) found greater suppression efficiency (reduction in oral intake per unit of food infused) in hyperphagic than normal, but the normals received a larger percentage of their caloric intake infused than the hyperphagics, and it is more difficult to get high suppression efficiences at high levels of infusion with water intake (Kissileff, 1969). This possibility has not been tested on food intake. The possibility that one mechanism of hyperphagia in the VMH-lesioned rat is an inability to respond to short-term satiety signals arising from the gastrointestinal tract with a normal response to signals which initiate feeding which continue to occur with the same frequency as before the lesion is still viable.

A second possible mechanism of hyperphagia expressed by the reduction in satiety ratio of the lesioned animal is that the VMH or its connections contains the mechanism responsible for maintaining satiety between meals. Sensitivity to satiety signals may be reduced causing the lesioned animal to eat before the level of a presumed satiety signal drops to as low a level as must occur in the normal animal. If blood glucose is conceived as such a signal, Steffens (1969) has shown that VMH-lesioned rats initiate meals at higher levels of plasma glucose than do normals. Another possibility is that the satiety signal itself disappears more rapidly in the lesioned animal than in the normal. This mechanism implies the existence of sensors which relate the size of a meal to the interval which follows it. While there is a correlation between the sizes of meals and the intervals which follow them (Balagura & Coscina, 1968; LeMagnen & Tallon, 1966; Snowden, 1969; Thomas & Mayer, 1968), the values (0.2-0.5) of the correlation

coefficients found in many animals suggest that this kind of mechanism can account only for 20 to 30 per cent of the variance in intermeal intervals from their means, and therefore the derangement of this mechanism could account only for a fraction of the hyperphagia.

The third possible mechanism for hyperphagia is suggested by the fact that most of the overeating in the lesioned rat occurs during the light phase. LeMagnen et al. (1973) have proposed that this effect and the consequent hyperphagia is secondary to a primary loss of nychthemeral periodicity in the normal cycle of lipolysis and lipogenesis. Lipolysis normally occurs during the day and inhibits feeding (LeMagnen & Devos, 1970). The VMH-lesioned animal no longer shows this lipolytic phase and therefore overeats.

If this were the cause of the overeating following VMH lesions, then it would be expected that when the VMH-lesioned rat becomes obese and its food intake returns to normal levels, that the increased feeding during the day should disappear. However, the normal pattern of predominantly nocturnal feeding does not return when the lesioned rat becomes obese (Becker & Kissileff, 1974; Kakolewski et al., 1971). Although food intake in the obese lesioned rat is within the normal pre-lesion levels, it is distributed equally between the light and dark phases (Becker & Kissileff, 1974; Kakolewski et al., 1971). While this does not directly refute the previous hypothesis concerning the hyperphagia following VMH lesions, it does suggest the possibility that the disruption in the normal pattern of predominantly nocturnal feeding and the hyperphagia are independent. This idea is supported by the recent work of Bernardis (1973) in which a disruption in the diurnal distribution of feeding was produced without hyperphagia in weanling rats with a lesion in the dorsomedial nucleus.

The progressive series of changes in meal parameters observed by Becker & Kissileff (1974) and described above suggests a fourth possible mechanism which might cause hyperphagia. Kennedy long ago proposed that the VMH functions in the long-term control of body weight by regulating food intake in response to a circulating metabolite (1950, 1953). Hoebel and Teitelbaum (1966) showed that less total weight gain and lower food intakes follow VMH lesions if the animals lesioned were already obese. They suggested that a stimulus correlated with obesity controls food intake by activating cells in the VMH. When some of the cells in the VMH are destroyed, a higher level of body weight is required to inhibit food intake by activating the remaining cells, and the animal overeats until it reaches that level. Recent data of Gold (1973) support this notion since there is a correlation between amount of tissue destroyed and weight gain. However, if there is a stimulus correlated with obesity which activates cells in the VMH, no one has any knowledge of what this stimulus is and how it acts on the VMH.

Methods of Identifying Putative Alterations in the Meal Pattern

If there is a critical alteration or alterations in meal patterns which may cause hyperphagia, it should be possible to identify it. There are at least three possible ways that this could be done. First, it may be possible to make small and discrete lesions and knife cuts in the area of the VMH and to assess both the changes in feeding behavior and total intake in these preparations. For example, Gold (1973) has recently compared lesion size and location with rate of body weight gain. It thus might be possible to assess the changes in meal-taking behavior which accompany the different rates of body weight gain resulting from lesions of different size and location in the area of the VMH. Second, it may be

possible to prevent operation of the putative critical alteration in the meal pattern from occurring. It must be recognized that the lesioned animal's ability to respond to this challenge by further altering its meal pattern does not automatically eliminate the alteration as a causative factor in the hyperphagia. Third, it is possible to closely observe the feeding behavior during increased food intake produced by such changes as decreased ambient temperature, lactation, insulin injection, genetic obesity to determine the alterations in feeding behavior which occur under these conditions, and if increased food intake is always expressed in the same way. It has already been proposed that some of the normal functions of the VMH may be depressed in the hyperphagic pre-hibernating animal (Mrosovsky, 1964), and this animal could be suffering what could be described as a transient lesion. Since VMH lesions do not increase the high level of food intake in both weanling (Bernardis, 1973; Bernardis & Skelton, 1965, 1967; Han, Lin, Chu, Mu ,& Liu, 1965; Kennedy, 1975) and lactating (Kennedy, 1953) rats, it is possible that the control in the VMH critical for the maintenance of normal food intake is temporarily depressed in these circumstances. Therefore, studies of meal taking in animals under these conditions will show to what extent the alterations in feeding behavior, which is the cause of overeating in the VMH-lesioned rat, are shared by other preparations which overeat.

Strategy of the Present Investigation

The following strategy was employed: First, the experiments of Becker and Kissileff (1974) were replicated using a solid diet. This replication was essential in order to establish that their findings were not restricted to a particular diet. This is important because previous

studies using a solid diet are not entirely in agreement with the findings of Becker and Kissileff (1974). Teitelbaum and Campbell (1958) observed increases in meal frequency in VMH-lesioned rats and Balagura and Devenport (1970) found a period of extended nibbling following the lesion. Brooks et al.(1946) found that hyperphagia and meal size increased gradually following the lesion. Experiment 1 shows that the findings of Becker and Kissileff (1974) are precisely reproduced with solid diets.

The next step was therefore to examine meal patterns in animals exhibiting increased intake without VMH lesions. A convenient subject for this work is the genetically obese rat (Zucker & Zucker, 1961). It was found that the "fatty" rats showed a similar pattern of food intake as the rats with VMH lesions, suggesting the possibility of a generalized loss of nychthemeral periodicity in hyperphagia.

To explore this possibility further, patterns of food intake were studied in the lactating rat (experiment 3) which increases food intake by approximately the same amount as the VMH-lesioned rat (Kennedy, 1953). In lactating rats, however, with the exception of one period of time,nychthemeral periodicity was retained. This experiment suggested therefore that hyperphagia and nychthemeral periodicity of eating are under independent neurological control. This possibility was supported by the fourth experiment in which the strategy was to prevent nychthemeral periodicity of eating from influencing intake by restricting animals to 12 hours of feeding. In this experiment, VMH-lesioned rats in the immediate post lesion dynamic phase overate even when restricted to 12 hours of access to food.

General Methods

In experiments 1, 2, and 3 continuous records of meal patterns were recorded on printout counters, and these data analyzed with digital computer programs. This method of automatically collecting data and the computer programs for its analysis were adaptable for use with solid and liquid diets.

Apparatus: Solid Diets

Meal patterns were collected using an eatometer whose design and circuitry has been previously described in detail by Kissileff (1970). This device consists of a small V-shaped trough with compartments at opposite ends. One compartment contains a photoresister and the other a small light bulb. A food pellet (45 mg. Noyes) rests in the trough interrupting the light beam. Each time a pellet is taken the light passes across the trough and activates the photoresister. Another pellet is dispensed into the trough from a mechanical feeder (Lehigh Valley Electronics, Fogelsville, Pa.; Ralph Gerbrands Co., Arlington, Mass.) blocks the light beam, and inactivates the photoresister. Food pellets are always available except during a delay in delivery which is one second in length. The control circuit was arranged so that if a pellet failed to be delivered in one second another was dispensed. Counts of the frequency of light beam breaking and frequency of delivery generally agreed within 1.0 percent, but differences were occasionally higher due to animals eating pellets before they reached the bottom of the trough and therefore did not interrupt the light beam. In all cases, the data used for computing the results were the number of deliveries, not the

number of light beam interruptions. Spot checks of the equipment at irregular intervals indicated the feeders were always delivering 98-100 pellets out of every 100 activations.

During the periods when feeding patterns were collected (with the exception of the period during lactation in experiment 4--for details, see housing, experiment 4) the animals were housed individually in high-walled (22.9 x 20.3 x 48.3 cm) boxes with a floor consisting of 0.4 cm-diameter stainless steel bars separated from each other by 1.28 cm (center to center) to reduce the possibility of coprophagia. The eatometer protruded from one side of the box so that the bottom of the trough was 1.3 cm above the floor of the box. In the same side a 1.3 cm diameter hole was drilled, 5.1 cm from the floor and 3.8 cm from the center of the eatometer trough. A stainless steel spout (bent at a 30^{o} angle to the horizontal plane with a 0.38 cm diameter hole) was positioned at the bottom of a 50 ml buret so that the tip of the spout was just outside the hole in the box wall. Water was continuously available from this spout throughout all the experiments.

Apparatus: Liquid Diets

Meal patterns were recorded using a drinkometer (Grason-Stadler Co., West Concord, Mass.) to detect licking.

While meal patterns were collected the animals (with the exception of the period during lactation in experiment 4--see housing, experiment 4 for details) were housed individually in living chambers measuring 19.1 x 23.5 x 20.3 cm with a floor consisting of 0.4 cm-diameter stainless steel bars, 1.2 cm apart (center to center) to reduce the possibility of coprophagia. Two holes 1.7 cm in diameter were drilled in

one wall 10.2 cm apart (center to center) and 5.1 cm above the chamber floor. A stainless steel spout (see description above) attached to a 50 ml buret was positioned so that its tip was centered outside each hole approximately 0.2 cm from the cage wall. The liquid diet (see diets) was continuously available from one spout and water from the other. The spout from which the diet was available was coated with enamel (Insl-x Products Corp., Yonkers, N.Y.) and connected to the drinkometer by an insulated wire soldered to the spout (Becker & Kissileff, 1974).

Environment

With the exception of experiment 2 (see environment--experiment 2 for specific details) one living chamber used for collecting meal patterns with solid diets or two of the living chambers used for collecting meal patterns with liquid diets were placed in a closed white-walled chamber measuring 76.2 x 61.0 x 91.4 cm with a 6-watt fluorescent light suspended just outside a 1.3 cm-thick lucite window at the top of the box. The ambient illumination at the chamber floor was 3-foot candles in the liquid diet cages and 5-foot candles in the solid diet apparatus (measured with a Weston model 614 foot-candle meter). Differences were due to position of the chambers in the white-walled box. Unless otherwise noted a 12h-12h light-dark cycle (lights on 7 A.M., off 7 P.M., local time) was employed. The transition from standard to daylight saving time and back was delayed until the transition could be made at a time when no experiments were being conducted.

The box in which the individual chambers were placed was ventilated at the rate of .311 cubic meters per minute. Temperatures during the experiments varied between 20-22^{o} C. Relative humidity did not exceed 50 per cent.

Diets

Two liquid diets were used. In experiments 2, 3, and 4 when the animals were fed a liquid diet, it was sweetened condensed milk (Borden's Magnolia Brand) diluted 3:1 by volume with tap water with vitamins and minerals added. This mixture has been previously used (Becker & Kissileff, 1974) and the amount of minerals added was based upon the data of Kemmerer, Elvehjen, Hart, and Fargo (1932), Greenstein, Otey, Birnbaum, and Winitz (1960) and McCoy (1949). The liquid diet used in experiment 3 also consisted of sweetened condensed milk diluted 3:1 with tap water. Since mineral requirements for animals during lactation are greater (Nelson & Evens, 1961), a more concentrated solution of minerals was added to the milk. Both liquid diets contained 3.17Kcal/ml (based on manufacturer's specifications). Table 1 contains constituents for both diets.

In experiments 1 and 3 the solid diet consisted of 45 mg Noyes pellets. Most of the pellets used in these experiments were from lot number 14643. The caloric concentration of the diet was 4.3 Kcal/g (based on manufacturer's 1972 specifications). The constituents of the pellets used in this experiment are given in Table 2.

Before and after the major experimental periods (see individual procedure sections) and unless otherwise specified, the animals were maintained on Purina laboratory chow pellets.

Data Collection

Liquid diets. Each day at the same hour food intake and water intakes were measured to the nearest 0.1 ml, and body weight was recorded to the nearest gram. The rather viscous milk diet tended to leak, and spillage was collected under mineral oil (light) in a small plastic reservoir below the spout. Spillage was measured and subtracted from the total

Table 1. Directions for Mixing Diets

1.	Borden's sweetened condensed milk (Magnolia Brand)	1 14-oz. can (300 ml)
2.	Water	95.0 ml
3.	Vitamins - Polyvisol brand (Upjohn)	0.6 ml
4.	Mineral mix	5.0

Mix the following with water to 500 ml total solution in a volumetric flask.[1]

	Normal Rats	Lactating Rats
Cupric acetate ($Cu(C_2H_3O_2)_2 \cdot 2H_2O$)	.045 g	0.18 g
Ferrous gluconate ($C_6H_{11}FeO_{14} \cdot 2H_2O$)	5.1 g	20.4 g
Manganese acetate ($Mn(C_2H_3O_2)_2 \cdot 4H_2O$)	0.78 g	3.12 g

Blend 4 main ingredients in a blender for about 1/4 minute at low speed.

[1]This is a modified form of the mineral mix suggested by Kissileff (1972) with the following modification. The minerals are dissolved in a volumetric flask containing 500 ml of water instead of 200 ml as previously suggested.

Table 2. Formula for Noyes Stock Pellet Diet, 1972

Ingredients	Amount, per cent
1. Laboratory animal food	65.0
2. Glucose	7.5
3. Dry Milk	7.0
4. Flour	6.5
5. Water	5.0
6. Gelatin	3.5
7. Zein	2.0
8. Stearic Acid	1.5
9. Acacia	1.0
10. Calcium Phosphate	1.0

Source: P. J. Noyes Co., Lancaster, N.H.
1972 specification

intake, and all computations of meal size are based on this corrected intake. Spillage was less than 1 ml per day as long as the temperature remained below 21° C. Also at this time, the spouts and burets were cleaned with Alconox to prevent growth of bacteria which causes spoilage of the diet. Spoilage was never detected during the experiments reported here.

Solid diet. Each day at the same hour the total number of pellets consumed in the previous 24 hours, water intake to the nearest ml, and body weight were recorded. While eating, the animals spilled some of the diet. Spillage varied by 0.2 to 4.7 g. per day and was collected in a pan located under the cage floor. At the same time intake measurements were recorded, the spilled food was separated from the feces, and if damp or saturated with urine, dried under an electric heater and then weighed. The spillage was subtracted from the total amount eaten to produce the corrected total intake, and all computations of meal size are based on this corrected intake.

Automatic Recording of Feeding Patterns

All electrical recording equipment was housed in another room and connected to the apparatus with cables which plugged into the experimental chambers.

Each lick at the spout containing the experimental diet or pellet dispensed advanced the counting element (food channel) of a dual channel printout counter (Sodeco model PL 203). Another counting element (time channel) was advanced every 6 seconds by a pulse from a synchronous motor, and thus registered accumulated time continuously to the nearest 0.1 min. When the animal began feeding the time channel printed and provided a

record of the accumulated time at the beginning of the meal (start time, T_B). The meal was considered over when no feeding occurred for 1 min. and then both the time and food channels printed simultaneously. This provided a record of the accumulated time at the end of the meal (stop time, T_E) and the number of licks or pellets consumed in that meal. Following each meal the food channel was reset to zero. The time channel continued accumulating.

At the same time each day that the intake data were collected, the total number of counts on the time channel was recorded, and then this channel was reset to zero. The time to the nearest minute was also recorded when this reset was made. Thus the number which is printed by the time channel at the beginning of the meal and the number printed at the end represented the accumulated time from the moment the counters were reset (Becker & Kissileff, 1974). In addition a separate impulse counter (Sodeco model TCeZ4E) accumulated the number of licks or pellets. This counter indicated the total number of licks or pellets taken each day and was reset to zero when its output was recorded. The purpose of this counter was to provide an independent measure of the events to be sure the printout counters were working reliably.

Data Analysis

Each bout of feeding during the day was specified by the three numbers described above: the number of licks or pellets in the bout (L or P); the accumulated time at the start of the bout T_B; and the accumulated time at the end of the bout T_E. From these three numbers meal size in ml or g, duration of the meal in minutes, the length of the interval between meals in minutes, and the local time of occurrence

of the meal to the nearest minute could be obtained. Indeed, any other conceivable information about the meal pattern exclusive of the events within the feeding bout could also be derived using these three numbers. Table 3 provides all the formulas used in calculating the information mentioned above.

Computer Programs

In order to facilitate processing of the large quantities of data collected, a series of computer programs were employed. Data collected with the printout counters were punched onto Hollerith cards for input to the computer and verified. From these cards the data were initially processed and transferred to a magnetic tape using the card-to-tape program described next, and this tape was then used as the input for a series of other programs which provided either a more intensive analysis of the data or a graphic display.

Card-to-tape program. For each day's data a deck of Hollerith cards was assembled with the information on each card arranged as follows. On Card 1 was punched the subject's identification, the date the data was collected, the experimental condition (e.g., prelesion, post lesion), subject's body weight, local time of day printout counter was reset, total accumulated on the printout counter time channel immediately before it was reset (NTIME), and the number 1 in specific separate columns if either the present day or the following day was not consecutive. Card 2 contained total intake in g or ml, spillage in g or ml, water intake in ml, and total numbers of responses (pellets or licks) from the independent accumulating counter. The cards following contained the bout sizes in licks or pellets punched right justified in consecutive 5-column fields

for each bout and continued until all bouts were exhausted. A field of 0 or a blank card, if all fields on the last card were filled, indicated the end of the set of cards specifying bout size.

The next set of cards contained start (T_B) and stop (T_E) times (see automatic recording of feeding patterns) for each meal punched right justified by meals sequentially in successive 5-column fields. The first start time of the next day was punched as the last number on the last card of this set. This number along with NTIME was used to compute the interval from the last meal on the present day to the start of the first meal on the next consecutive day. (See Table 3 - by adding NTIME to the equation $T_{BC} - T_{EPs} + 1$ for calculating I_F) Since data were processed sequentially by day and the subsequent day's data were read into the same storage areas as the present day's data, this interval was stored in a special location and used as the interval preceding the first meal of the subsequent day before it was written over. The end of the set of cards containing the start and stop times was indicated in the same way as it was for cards containing meal sizes. If the present day was not consecutive, but the time at the end of the last feeding bout on the previous day was known, this time could be included by the addition of an extra card following the regular cards on which the stop time of the last feeding bout and NTIME from the previous day were punched. The last card in each day's deck was used to indicate (by 1 or 2 in the first column) whether or not the present experimental condition was to be continued or terminated. Decks for each day were arranged sequentially and preceded by a card which specified the type of data (e.g., solid or liquid diet), the number of experimental conditions to be analyzed, and the caloric value of the diet.

Using the card-to-tape program the computer read these cards and with the formulas given in Table 3 calculated the following values: meal size

Table 3. Computations made from Raw Data

MS (liquid diets)	$L \times N_T/N_F$
MS (solid diets)	$P \times .045 - S \times \frac{P}{N_P}$
N_T (solid diets)	$N_P \times .045 - S$
D	$T_E - T_B - 1$
I_F	$T_{BF} - T_{EPs}$ 1
I_{Pc}	$T_{BPs} - T_{EPc}$ 1
H_{Ps} : M_{Ps} *	
R_{Ps_c}	I_{Pc}/MS_{Ps}
R_{Ps} (satiety ratio)	I_F/MS_{Ps}

KEY:

MS	meal size (ml or g)	B	beginning of meal
L	number of licks in meal	Ps	present meal
P	number of pellets in meal	Pc	preceding meal
S	spillage	F	following meal
N_T	total food intake in day (ml or g)	I	intermeal interval
N_P	total pellets in day	H	hour (real time)
N_F	total number of licks in day	M	minutes (real time)
D	duration of meal (min)	h	hour when counters were reset
T	elapsed time (min)	m	minute when counters were reset
E	end of meal	R	interval:size ratio

Thus, I_{Pc} means the interval preceding the present meal, I_F means the interval following the present meal. T_{EPs} is the elapsed time at the end of the present meal.

*H and M are computed by (1) dividing T_{Ps} by 60 and eliminating the remainder (r); (2) adding this quotient to h; (3) r is then added to m to obtain M; (4) if r + m (total) exceeds 60, an additional hour is added to H and the difference between 60 and total becomes M.

in ml or g, the duration of each meal in minutes, the interval before and following each meal in minutes, and the local time of day to the nearest minute that each meal began. A check routine in the program determined that the input and the calculations derived from it were internally correct. Error messages (diagnostics) appeared during the program execution if the following conditions occurred: unequal number of meal sizes and pairs of start and stop times; total number of licks or pellets for all meals not equal to total number of licks and pellets recorded on accumulating counter, durations of individual bouts greater than 20 minutes (a condition which was noted empirically to indicate an error in key punching except under unusual conditions); and that each succeeding start and stop time was greater than the number which preceded it (with the exception of the first start time for the following day).

The values calculated along with information directly from the data cards were written on a magnetic tape. The data on the magnetic tape were arranged in the following manner. For each day's data there were eight individual records. The first record contained the subject's identification, the date the data were collected, total intake in g or ml, total number of feeding bouts during the day, two integers 1 in separate words to indicate if the present or following days were not consecutive, and in another separate word the integer 1 to indicate whether another day's data were to be included in the experimental condition, or 2 to indicate that the present day was the last in the experimental condition. The second record contained the number of the present day in the experimental condition, the caloric concentration of the diet, the weight of the subject, the hour and minute in local time that the present day began (actually the time the

printout counters were reset on the previous day), NTIME, the length of the day in hours and minutes (the data were not collected at exactly the same time each day and hence the length of the day varied), and the type of data (e.g., liquid or solid). The third record included the water intake in ml and the intake-to-lick ratio for liquid diet and the water-to-food ratio for solid diet. Each of the next five records consisted of a list of values written in the same order as the feeding bouts occurred with a value for each bout. The first of the lists (fourth record) was composed of the hour during which each feeding bout began using a 24-hour clock. The next list (fifth record) consisted of the minute each individual bout began. On the sixth record was written a list of the feeding bout sizes in g or ml (to the nearest .001), and on the seventh record there was a list of the duration (to the nearest 0.1 minute) of each individual bout. The last record contained a list of the intervals (to the nearest 0.1 minute) following each bout.

The data for each day were written sequentially in the manner described above on the tape. Following the data for the last day in an experimental condition, a mark was placed on the tape to indicate an end of a file. Thus each experimental condition comprised a separate file on the tape. A separate list of the experimental conditions and the order in which they were written on the tape were maintained. The data for any condition could easily be accessed by moving the tape forward a specified number of end-of-file marks. Before entering new data the tape was moved forward ahead of all existing files.

The other three programs described below used the tape generated by the card-to-tape program as the input source.

Program for the Distribution of Intermeal Intervals. The purpose of this program was to draw a frequency distribution of the lengths of the intervals between feeding bouts.

The tape was advanced to a particular file which contained the data from the experimental condition to be analyzed. The records for the first day's data in the experimental condition were read. The intervals between the feeding bouts were then placed into class intervals according to their length in time. The size of the class interval was five minutes for intervals between feeding bouts of less than 40 minutes in length and 20 minutes for intervals which were 40 minutes or longer in length. The first class interval contained all intervals of 5 minutes or less in length; the second, all intervals more than five minutes, and up to 10 minutes in length; and so forth until the length of intermeal intervals reached 40 minutes. The the next class interval included all intervals greater than 40 minutes, but less than or equal to 60 minutes, etc. up to 400 minutes. The last class interval (380-400 minutes) also included all intervals greater than 400 minutes. Then the data for the second day in the experimental condition were read and the intervals from this day placed into the proper class intervals. This process was continued until the data from all days in the experimental condition had been placed in the proper class intervals, the number of intermeal intervals in each class interval was divided by the number of days in the experimental condition to produce the mean number of intervals of each particular length that occurred per day. In addition the percentage of the total of all intermeal intervals for each length was calculated.

The results of these calculations were then plotted. The size of the intermeal interval in minutes was plotted on the abscissa and the mean number per day on the ordinate. Eight points, each representing 5-minute intervals in length, were plotted up to 40 minutes. One point was plotted for each 20-minute interval from 40 to 400 minutes. In reconstructing the plots for display the number of intervals greater than 260 minutes have been added to the 240-260 minute class interval. Each point represented

the mean daily number of intervals of less than or equal to its corresponding abscissa value, but greater than the preceding abscissa.

Program for Meal Pattern Profile. The purpose of this program was to provide a visual record of the daily temporal pattern of food intake to aid in the qualitative examination of the effects of different manipulations upon these patterns.

The tape generated by the card-to-tape program was used as the source of the information upon which daily meal pattern profiles were drawn with an on-line plotter. This tape was first advanced a specified number of end-of-file marks to a particular file which contained the experimental condition from whose data meal pattern profiles were drawn. A Hollerith card containing the hour of the day (using a 12-hour clock) the lights were turned off, and the criterion for defining intermeal intervals to be used in the profiles was read. For each 24-hour period a single line was drawn to represent the temporal pattern of feeding. The line began at the left which represented the time the lights were turned on and continued across and terminated on the right which represented the time just before the lights were turned on. Since all animals in this investigation were on a 12h - 12h light-dark cycle, the midpoint in the line corresponded to the time the lights were turned off. Hence to the left of the mid-point was shown the temporal pattern of feeding during the light phase, and to the right the pattern during the dark phase. When no feeding occurred, a straight line was drawn. When the animal began eating, the trace was elevated from the baseline to a height scaled to the amount consumed. If no additional feeding occurred within the period of time specified by the criterion for defining the intermeal interval, the trace returned to the baseline. If more food was consumed within this period, the trace remained

above the baseline until the time that the additional feeding began and then moved upward to show the amount consumed. Thus, for example, if a 20-minute criterion was employed, the trace would remain above the baseline and continue moving upward as long as the animal continued to eat, and would not reset unless there was no additional feeding for at least 20 minutes. The length of time the trace remained elevated indicated the duration of the meal.

Program for Meal Pattern Data (MPP). The purpose of this program was first to provide a detailed quantitative analysis of each day's meal pattern and second to calculate the mean values of all meal parameters over the entire experimental condition so that comparisons between experimental conditions could be made.

A Hollerith card upon which was punched the hour the lights were shut off (using a 12-hour clock) and the criteria for defining intermeal intervals to be used in the analysis of the data was read. The tape generated by the card-to-tape program was advanced a specified number of end-of-file marks to the particular file which contained the experimental data to be analyzed using this program. The records for the first day in the experimental condition were read. The data were then analyzed in the following way with each of the criteria listed on the Hollerith card described earlier. The use of different criteria to define intermeal intervals resulted in changes in the lists of bout sizes, bout durations, and the intervals between bouts which had been read from the magnetic tape. In order to produce the changes in these lists the following procedures were used with each criterion. The interval following every feeding bout was examined. If this interval was greater than or equal to the criterion, there were no changes made. If the

interval was shorter than the criterion, the present and succeeding feeding bouts were considered one meal; their sizes added together, and their durations and the interval between them added together. Since a bout, a duration, and an intermeal interval were eliminated, all succeeding values were moved upward one position in the list.

For each criterion a separate page was printed containing the following information on each individual feeding bout: the size of the bout in g or ml, the duration of the bout (to the nearest 0.1 minute), the size of the bout in calories, the interval to the next feeding bout (to the nearest 0.1 minute), the interval from the last feeding bout (to the nearest 0.1 minute), and the satiety ratio (Becker & Kissileff, 1974; Panksepp, 1973). The satiety ratio was calculated by dividing the size of the interval to the following feeding bout in minutes by the size of the present bout in calories. On the bottom of the page was printed the mean of each of these values for the entire day and the mean individually for the light and dark phases. These means were stored in separate locations for use in calculating the mean value of these parameters for the entire experimental condition. This procedure continued until all of the data from all of the days in the experimental condition were read.

Following the individual daily analysis of the data, the program then averaged the meal pattern parameters for the entire experimental condition by dividing the sum of the daily mean by the number of days in the condition. Mean daily intake and mean intake during the light and dark phases, as well as the mean per cent of the total intake consumed during the light and dark phases, were first calculated. Then the total number of meals each day and the number of meals occurring in the dark and in the light were averaged.

The mean daily meal size was computed for the whole day and separately for the light and dark phases. In addition, the duration, the length of the intervals following meals, and the satiety ratios for all meals and separately for dark phase meals and light phase meals were averaged for the entire condition.

Surgical Procedures

In experiments 1 and 3 all lesioned rats had chronic platinum electrodes implanted in the VMH under barbiturate anesthesia, and given at least one week to recover from surgery before any experimental manipulations were begun. When lesioned, they were briefly anesthetized with ether.

Electrodes. 0.015 inch-diamter (.38 mm) straightened platinum-10 per cent iridium (Mathey Bishop Platinum Works, Malvern, Pa.) wire was cut to 7 mm length and inserted into 22 ga. stainless steel tubing (Hoebel, 1964) cut to 18 mm lengths so that the platinum extended 3 mm out of the tubing. The platinum and stainless steel were insulated with enamel (Insl-x Products Corp., Yonkers, N.Y.) to 0.5 mm from the tip.

Surgery. The electrodes were implanted bilaterally, sterotaxically into each animal under Nembutal (sodium pentobarbital) anesthesia (43 mg/kg). The animal was positioned with skull flat and coordinates were 6 mm anterior to the eat bars of the stereotaxic instrument, 0.75 mm lateral to the midsagital sinus, and 8.5-8.8 mm below the dural surface. Prophylactic antibiotics (60,000 Units penicillin and 10 mg Terramycin) were given intramuscularly in separate depots, immediately after surgery.

Lesioning. When animals were lesioned, they were removed from their living chambers, anesthetized with ether, and then lesioned by passing an anodal DC current of 3 ma (rectal cathode) through each electrode for 40

seconds. Immediately following lesions all animals were returned to their living chambers and given ad libitum access to their diet.

Sham Implants and Lesions. In experiment 1 all sham-lesioned rats had sham implants. The sham implant consisted of two 22 ga. stainless steel tubes cut to 18 mm lengths and implanted bilaterally stereotaxically at the same coordinates as the lesioning electrodes. Sham implants, however, were placed just below the dural surface. Surgical procedures were completely similar to VMH electrode implants.

Since the sham implant appeared to have little effect upon the behavior studied (cf. Table 16), only some of the animals in experiment 3 were sham implanted.

All animals classified as sham lesioned (both implanted and not implanted) at the time of the sham lesion were removed from their living chambers, anesthetized with ether for a period of time approximately equivalent to that necessary to make a VMH lesion (3-5 minutes), and returned to their living chambers and given immediate ad libitum access to their diet.

Histology

Following the experimental procedures, all animals were sacrificed with an overdose of sodium pentobarbital and perfused with a solution of 10 per cent formalin in isotonic saline. The brains were removed from the skull and embedded in celloidin. Brains were sectioned at 40 microns on a freezing microtone. Alternate fifth sections were stained with a cresyl violet and Weil stain. Sections were examined with the aid of a microprojector and lesion extent was estimated with the aid of the atlas of Konig and Klippel (1963).

EXPERIMENT 1. Effects of VMH Lesions on Meal-Taking With a Solid Diet

Investigations of the immediate effects (Balagura & Devenport, 1970; Becker & Kissileff, 1974; Brooks et al., 1946) and more permanent alterations (Becker & Kissileff, 1974; Teitelbaum & Campbell, 1958) in feeding behavior following VMH lesions have yielded different results.

Brooks et al., (1946) suggested that both meal size and food intake gradually increased following the lesion. A later study (Balagura & Devenport, 1970) found immediate hyperphagia characterized by an initial disruption of meal-taking in which there was a period of prolonged nibbling lasting about nine hours. Becker and Kissileff (1974) found that a discrete meal more than twice the size of any previous meal occurred within minutes following the lesion. There are several possible explanations for these discrepancies. The procedures employed by Becker and Kissileff (1974) were different from the earlier reports in at least three respects:

1) In the earlier studies (Balagura & Devenport, 1970; Brooks et al., 1946; Teitelbaum & Campbell, 1958) the animals received all the necessary surgery on the same day as lesioning. It is possible that the trauma from the surgery combined with the effects of the barbiturate anesthesia could have effected the initial response to the lesion. Becker and Kissileff (1974) lesioned their animals through implanted electrodes, anesthetizing the animals for only a brief period with ether. The extensive implantation surgery occurred at least one week prior to the lesion.

2) It is also possible that the differences observed were the result of differences in the metal of the electrodes. Becker and Kissileff (1974) lesioned their rats using platinum electrodes while Balagura and Devenport (1970) used stainless steel electrodes. While there have been studies suggesting that lesions made with platinum are less effective in producing obesity (Rabin & Smith, 1968), other investigators (Hoebel, 1965; Gox, Kakolewski, & Valenstein, 1969) have produced obesity with platinum at least in female rats. However, Larkin (1973) has shown that although rats lesioned with either platinum or stainless steel become equally obese, only those rats lesioned with platinum electrodes increased bar pressing for food on FR-64 schedules without pre-operative training. It is therefore possible that while lesions made by stainless steel and platinum both produce obesity, there might be subtle differences in the feeding behavior which accompanies the hyperphagia.

3) Finally, it is also possible that the inconsistency in results could have been caused by the different diets used since they varied both in palatability and texture. Brooks et al., (1946) and Balagura and Devenport (1970) both used solid diets while Becker and Kissileff (1974) used a more palatable liquid diet. There is some evidence to suggest that the nature of the diet might be important since VMH lesions are known to cause finickiness (Corbit & Stellar, 1964; Teitelbaum, 1955). Furthermore, there are quantitative differences in the way lesioned rats consume different diets (Teitelbaum & Campbell, 1958). Hyperphagia is accompanied by larger meals when animals are fed a liquid diet, but when animals are fed a solid diet, meal size is not

elevated as much, and the daily number of meals is increased, although not significantly (Teitelbaum & Campbell, 1958). This difference could be due to the criterion used to define intermeal intervals. Teitelbaum & Campbell (1958) arbitrarily selected an interval of five minutes as the criterion to define intermeal intervals. Thus, using this criterion, if an animal were eating, paused for 5 minutes, and then started eating again, it would have been considered that the animal took two meals. Such pauses may not be indicative entirely of natural satiety, but rather the result of disruptions caused by the activation of other motivational systems such as drinking within the meal (Kissileff, 1970). If an animal is eating extremely large meals on a solid diet, it is possible that several such pauses could occur. Kissileff (1970) has suggested that in the analysis of meal pattern data the criterion used to define intermeal intervals and separate meals be selected on a rational basis (nadir of pauses in distribution of feeding bouts which corresponds to the intermeal intervals which normally occur least frequently) and second that the data be examined with several different criteria to determine that the selected criterion did not bias the results.

As described in the general introduction, the findings of Becker and Kissileff (1974) that hyperphagia is expressed as an immediate increase in meal size and equal distribution of feeding during the dark and light phases have important implications for explanations of the mechanism of hyperphagia in the rat with VMH lesions. However, unless it can be demonstrated that these results are not simply restricted to a particular diet their importance as a general explanation

is greatly diminished. The major purpose of the present experiment was to provide this extension by demonstrating that the findings of Becker and Kissileff (1974) also hold with solid diets. In addition, this experiment will also determine whether dietary differences account for the difference in the initial effects of VMH lesions seen by Becker and Kissileff (1974) and Balagura and Devenport (1970). Finally we shall attempt to explain the differences in meal patterns seen by Teitelbaum and Campbell (1958) when rats were fed either solid or liquid diets.

Method

Continuous records of meal-taking patterns were obtained from rats eating 45 mg Noyes pellets (see General Methods, apparatus: solid diet) before electrodes were chronically implanted in the VMH, after implantation of electrodes, for 20 consecutive days after the lesions were made through the electrodes, and from the 65th through 75th days following the lesion. The patterns were then analyzed using the computer programs described in General Methods. The following parameters of meal-taking were examined; light-dark distribution of daily total intake, mean daily meal size, and the mean daily number of meals. Meal pattern profiles were obtained for each animal. Histogram distributions of intermeal intervals were also obtained.

Animals

Six naive female Wistar rats (Carworth Labs, New City, New York) were used. The animals weighed 261 to 316 g at the beginning of the experiment. Four had chronic electrodes implanted in the VMH (see

General Methods, electrodes and surgery) and subsequently were lesioned (see General Methods, lesioning). The other two animals had sham implants and were used as operative controls (see General Methods, sham implants and lesions). As a result of the weight gain in the first 30 days following the lesion 3 of the 4 lesioned rats were classified as obese (weight gain of greater than 140 g in 30 days) and one animal as moderately obese (weight gain of greater than 100 g, less than 135 g in 30 days). These criteria are identical to the 30-day weight gain criteria used by Becker and Kissileff (1974) and, while arbitrarily chosen, have shown that rats which fall into the obese category possess all of a constellation of disturbances (increased meal sizes, decreased satiety ratios, increased light phase intake) while animals which are only moderately obese show only fragments of the hyperphagia syndrome. Table 4 shows all the data used in making these classifications as well as the 60-day weight gains which will be discussed later.

Sequence of Procedures

Body weight and water intakes were measured daily for 3-5 days and in addition meal patterns were recorded (see General Methods, automatic recording of feeding patterns). Electrodes were then implanted (see General Methods, surgical procedures). After a recovery period of at least one week, meal pattern data were recorded, and body weight and water intakes were collected for 5 consecutive days. Then at the usual time of collecting the daily measurements, the animals were anesthetized, lesioned (see General Methods, lesioning) or sham lesioned (see General Methods, sham implants and lesions),

Table 4. Body Weight Parameters

Animal	Wt. at Lesion (g)	30 Day Weight Gain (g)	60 Day Weight Gain (g)
		Obese	
DD 3	331	198	283
DD 7	298	277	344
DD 9	288	150	188
Mean	306	208	272
		Moderately Obese	
DD 8	282	101	141
		Sham Lesion	
DD 4	305	-13	23
DD 5	279	8	24
Mean	292	-2.5	24

returned immediately to their living chambers, and given ad libitum access to food and water.

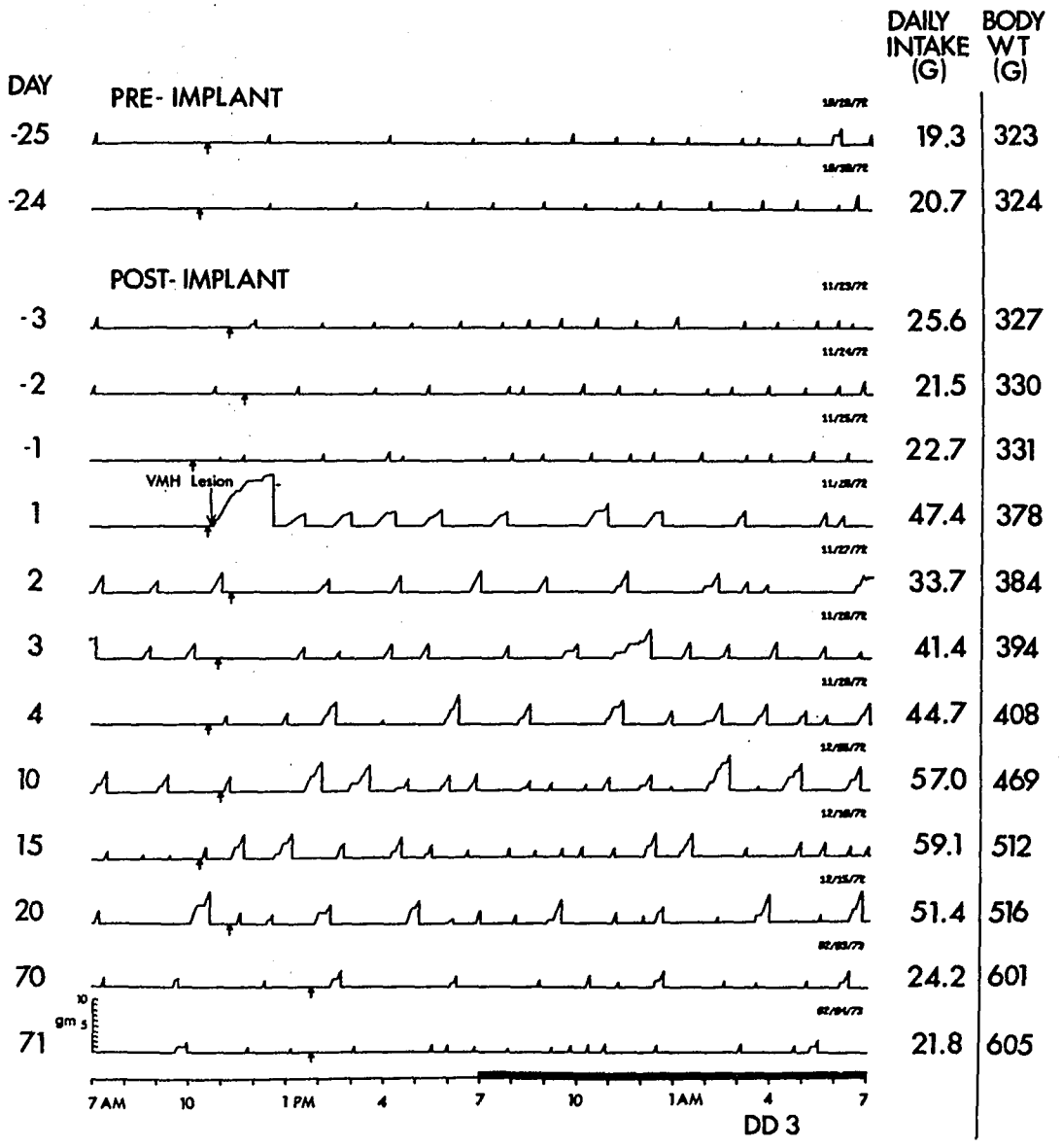
Meal pattern data, body weight, and water intake measurements were then collected for 20 consecutive days. On the 20th day following the lesion the animals were placed in individual mesh-bottom cages measuring 24.5 x 18 x 18 cm with ad libitum access to Purina laboratory chow pellets (placed on the cage floor) and water. Body weights were measured every fifth day. On the 65th day following the lesion the animals were returned to the cage in which meal patterns were previously recorded and measurements of body weight and water intake were collected in addition to meal pattern data for 10 consecutive days.

Following a series of procedures to be reported separately lasting approximately three months, the animals were sacrificed (see General Methods, Histology).

Results

The major results of this experiment were as follows. First, immediate hyperphagia within minutes after lesion was observed as a discrete meal larger than any previous meal. Second, the normal nychthemeral pattern of predominantly nocturnal feeding was immediately replaced by equal intakes during the light and dark phases. Third, in all animals there were sequential changes in meal parameters as body weight increased although these changes varied in individual animals (see Figure 1). In addition to these findings, the distribution of intermeal intervals was dramatically altered following the lesion, and the frequency of brief interruptions between feeding bouts was

Figure 1. Meal pattern profile of a representative lesioned rat (DD 3) drawn by a plotter from data input to computer (See General Methods - meal pattern profile program). Each line shows temporal pattern of feeding for an entire 24-hour period. Elevation of trace above base line indicates feeding. Amount taken is indicated by height of trace above baseline (see scale, lower left). Duration of meal is indicated by length of time (across horizontal axis) trace remains elevated. These records were constructed using a 20-minute criterion for inter-meal interval. That is, trace remained above the line and continued moving upward as long as animal continued to eat with pauses of less than 1 min. and did not reset unless there was no additional eating for at least 20 min. For example, in the thirteenth meal on line 1, animal paused for a few minutes and then continued eating. This is indicated as a horizontal line within that meal. The arrow beneath each line indicates when measurements of food and water and body weight were made. Intakes for the dated period extending between the arrows from one line to the next are shown at the right; body weight at the end of each period is also shown.


DAY
DAILY INTAKE (G)
BODY WT (G)
PRE- IMPLANT
-25
19.3
323
-24
20.7
324
POST- IMPLANT
-3
25.6
327
-2
21.5
330
-1
22.7
331
VMH Lesion
1
47.4
378
2
33.7
384
3
41.4
394
4
44.7
408
10
57.0
469
15
59.1
512
20
51.4
516
70
24.2
601
71
gm
10
5
21.8
605
7 AM
10
1 PM
4
7
10
1 AM
4
7
DD 3

greatly increased. The results will be presented describing the meal patterns at each stage of the experiment and will be preceded by a general description of the distribution of intermeal intervals and the rationale for choosing the 40-minute criterion to describe most of the results. The effect on the results of using other criteria for separating meals will be shown and the interpretation of the results using the various criteria will be discussed.

Distribution of Intermeal Intervals

In order to interpret the interruptions in feeding which separate discrete meals from each other, histograms were constructed in which the frequency of occurrence of interruptions in feeding for various lengths of time (i.e., the magnitude of intermeal interval) are plotted against the magnitude of intermeal interval. To express the bimodal nature of the distribution, the number of intervals within five minute time blocks up to 40 minutes and 20 minute blocks thereafter was counted and plotted. This method of breaking up the data permits grahic expression of the natural distribution of the frequency of occurrence of intermeal intervals of various sizes and enables us to describe the peaks and valleys in the distribution with precision.

Pre-Implant. Before the implantation surgery three (DD 3, 5, 9) of the six animals had a distinct bimodal distribution in the frequency of occurrence of intermeal intervals with a small peak at 1-5 minutes (mean of .67 intervals per day (Range=0.33-1.00) and containing a mean of 4.6 (R=2.1 - 7.3) percent of all intermeal intervals per day (see Figures 2 and 3). Extending this peak to include 5 - 10 minute intervals increased the number of intervals to a mean of .78 (R=.33 -1.00) per day which

Figure 2. Mean daily distribution of intermeal intervals per day for the 4 lesioned animals. Each point is the mean daily number of intervals exceeding the abscissa value of the preceding point and less than or equal to its own abscissa value. For example, the 60-min point is the number of intervals from 40.1 min to 60.0 min. Eight points, each representing 5-min intervals in length are plotted up to 40 min. One point is plotted for each 20-min interval from 40 to 260 min. The last point includes the number of intervals exceeding 260 minutes.

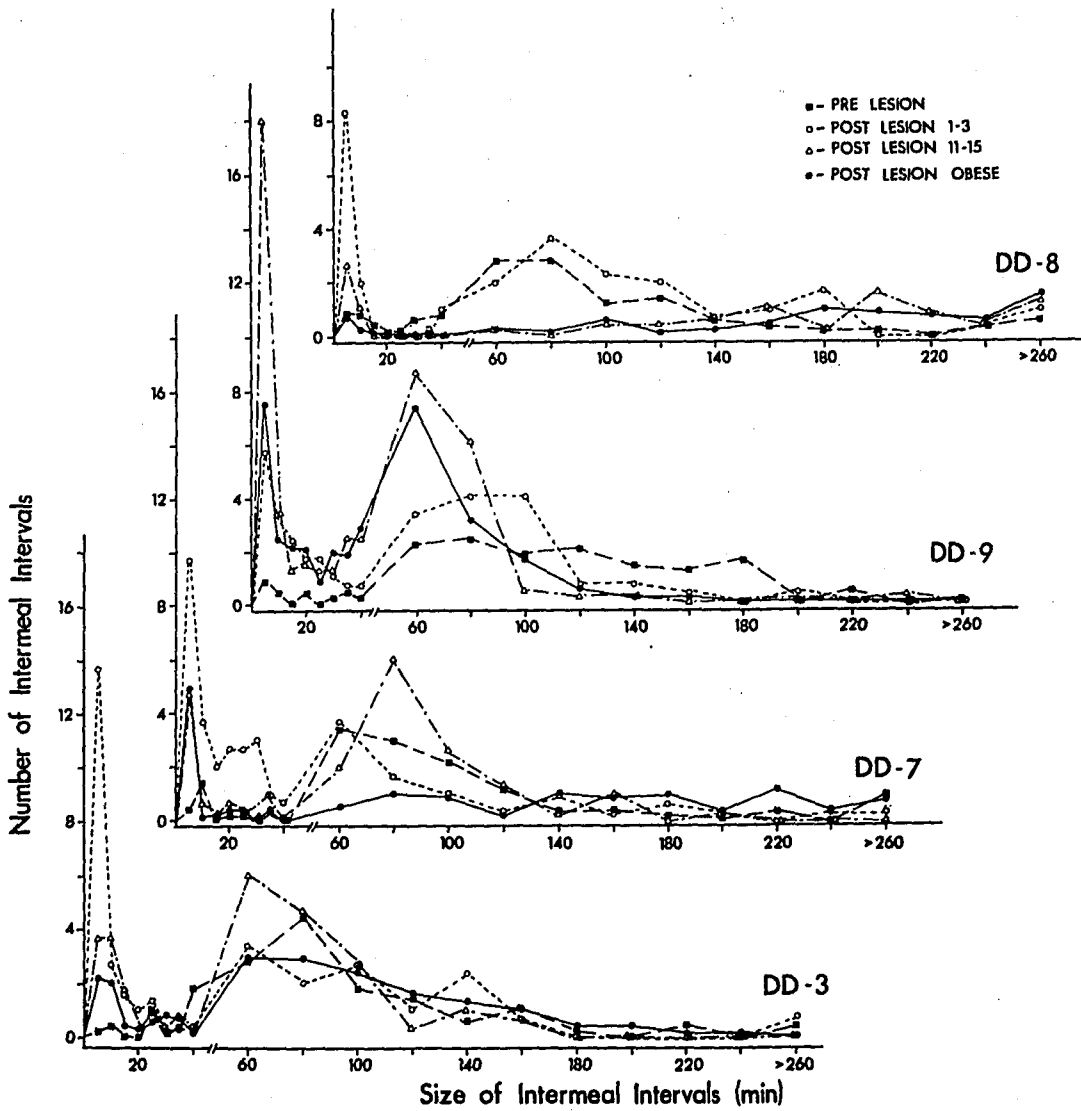
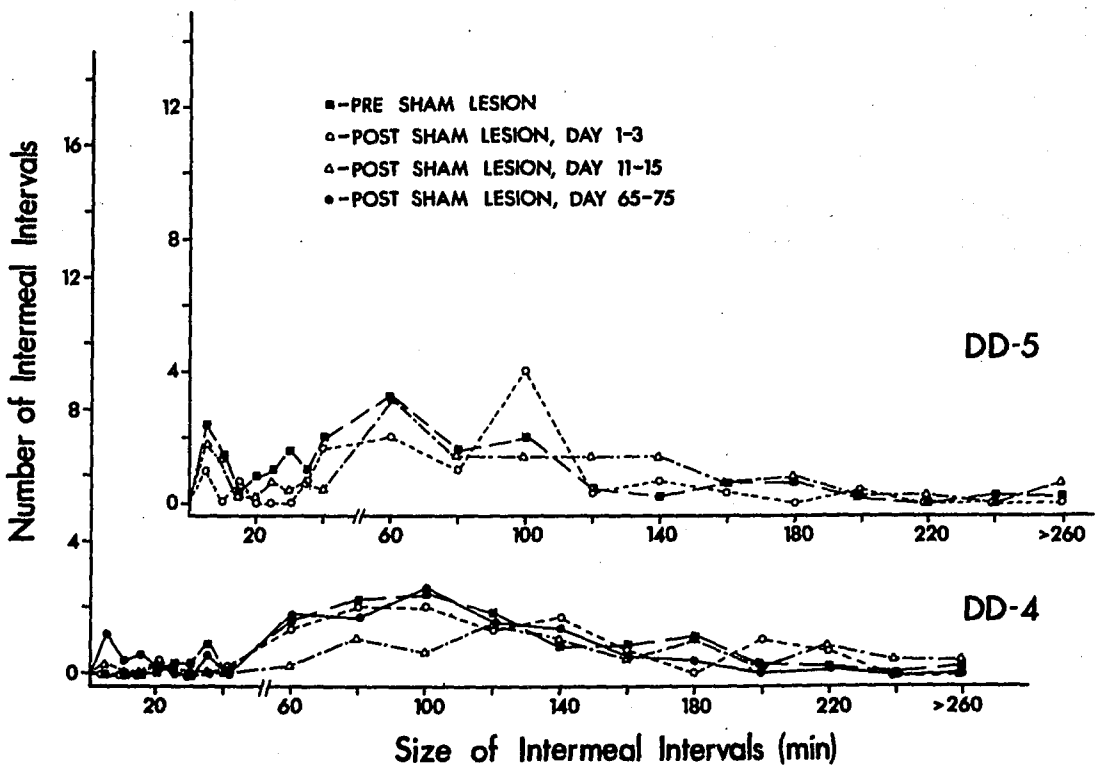

● - PRE LESION
○ - POST LESION 1-3
△ - POST LESION 11-15
● - POST LESION OBESE
DD-8
DD-9
DD-7
DD-3
Number of Intermeal Intervals
Size of Intermeal Intervals (min)
0
4
8
12
16
20
60
100
140
180
220
>260

Figure 3. Distribution of the mean daily number of intermeal intervals for the two sham-lesioned animals (DD 4, DD 5). For detailed description, see Figure 2.


■-PRE SHAM LESION
○-POST SHAM LESION, DAY 1-3
△-POST SHAM LESION, DAY 11-15
●-POST SHAM LESION, DAY 65-75
DD-5
DD-4
Number of Intermeal Intervals
Size of Intermeal Intervals (min)
20
60
100
140
180
220
>260

equaled a mean of 5.3 (R=2.1-7.3) percent of all intervals. There was a much broader peak in the distribution extending from 40 to 140 minutes (see Figures 2 and 3). A mean of 8.7 (R=7.2-11.0) meals per day which equaled 63.9 (R=56.5-76.6) percent of all meals were begun within 40-140 minutes following a previous meal. In the other three animals (DD 4, 7, 8) there was no small peak in the distribution of intermeal intervals between 1-5 minutes, but the broad and large peak extending from 40-140 minutes occurred (see figures 2 and 3). In these rats a mean of 8.2 (R=3.0-14.7) meals per day were initiated following intervals between 40 and 140 minutes in length. This equaled a mean of 52.7 (R=40.0-59.8) percent of all meals. In all rats there was a wide trough of least frequency occurring intervals between 10-40 minutes separating the two peaks (see Figures 2 and 3). A mean of 1.4 (R=0.6-2.3) meals per day or a mean of 10.9 (R=4.9-18.2) percent of all meals began following intervals between 10 and 40 minutes in length. In summary, all rats interrupted the majority of meals with intervals ranging from 40-140 minutes, and infrequent pausing occurred within meals defined by the 40-minute intermeal intervals. A natural break between meals was provided by the infrequent reinitiation of eating within 10 to 40 minutes after eating had stopped. In other words, using a 5-minute criterion as opposed to a 40-minute criterion would add 1 meal a day on the average.

Pre-Lesion or Sham-Lesioning Procedures. Following the implantation procedure or the sham-operation procedure all of the animals except two, DD-4 and DD-3, continued to display a clear bimodal distribution

in the frequency of occurrence of intermeal intervals (see Figures 2 and 3). The smaller peak occurred at 5 minutes (mean of 1.1 (R=0.4-2.4) intervals per day which equal a mean of 6.9 (R=2.6-11.5) percent of all intermeal intervals). The inclusion of the 5-10 minute intervals in this peak increased the number of intervals to a mean of 12.3 (R=7.8-18.3) percent. In DD-4 and DD-3 the smaller peak disappeared after the surgery. In all the animals there was a broad peak in the distribution extending from 40 to 140 minutes with its maximum at 40-60 minutes or 60-80 minutes. A mean of 56.1 (R=41.3-67.5) percent of all meals or a mean of 11.6 (R=8.8-14.8) meals per day were initiated within 40-140 minutes following a previous meal. The least frequent intervals between bouts of eating were between 10 and 40 minutes and together comprised only a mean of 14.4 (R=9.1-29.8) percent of all the intervals or a mean of 2.6 (R=12.0-6.6) intervals per day. In summary following the surgical procedure there were a mean of 3.4 more intervals per day in the longer range (40-140 min) and a mean of 1.2 more intervals of less than 40 minutes per day.

Post Lesion or Sham Lesion. Immediately following the lesion (day 1-3 post lesion, open circles, see Figure 2) the distribution of intermeal intervals became more sharply bimodal in all lesioned rats as a result of a 9.6 fold increase in the number of shorter (1-10 minute) interruptions to a mean of 12.5 (R=10.0-16.3) intervals per day. The percentage of intervals of less than 10 minutes rose to a mean of 41.1 (R=29.9-49.0). In all of the lesioned rats the general shape

of the broad peak of longer intervals remained unchanged (mean of 10.3 (R=7.7-12.7) intervals per day, mean of 41.9 (R=21.9-64.0) percent of all intervals). Finally in all of the lesioned rats there was an increase in the number of normally least frequent intervals (10 to 40 minutes) to a mean of 6.8 (R=1.0-12.0) meals per day, although these remained the least frequent intervals comprising only a mean of 19.2 (R=4.1-26.7) percent of all the meals. Most of this increase was in the shortest intervals in this range. The number of intervals between 35-40 minutes did not increase (see Figure 2). In summary the first three days of the post lesion period was characterized by a large increase in the intermeal intervals of shorter frequency but no change in the frequency of the longer ones.

During subsequent periods, the sharply bimodal distribution of intermeal intervals remained in all lesioned animals but the magnitude of the peak at 1-5 min was greatly reduced except in DD9 where it became greatly elevated between 1-5 minutes during days 11-15 post lesion. When the animals became obese or in the case of DD 8 stopped gaining weight at a moderate level of obesity, there was in all animals except DD 9 a characteristic flattening of the broad peak. DD 9 continued to have a sharp peak in the number of intermeal intervals between 40 and 140 minutes (see Figure 2).

In the sham-lesioned rats the distribution of intermeal intervals was not systematically affected by the anesthetization (see Figure 3) and remained quite stable on subsequent days demonstrating that changes in this distribution in the lesioned rats were not simply due to random fluctuations but were the consequence of the production of brain damage.

Criterion. Kissileff (1970) suggested that in the absence of psychophysical data on satiety that the nadir in the distribution of intermeal intervals could be used as an indication of a period of time following a meal when satiety is high. The rationale behind this is that meals should be separated from each other by periods of satiety, and a time when an animal is least likely to eat, may be a period when satiety signals are at their maximum (if such a continuum in satiety signal level exists). It is clear from Figure 2 and the exact values presented above that following VMH lesions the valley in the bimodal distribution of intermeal intervals which normally extends from 10 to 40 minutes is replaced by a more gradual decline in the number of intermeal intervals from 10 to 40 minutes with a nadir at 40 minutes in DD 3, 7, and 9. It may be coincidental that these were also the same three animals which became obese. DD 9 which became less obese displayed a nadir in intermeal intervals in the first post lesion period (day 1-3) at 20 minutes. In view of these individual differences and the quantitative differences in meal sizes depending upon the criterion chosen to define meals, the results are described quantitatively using the 40-minute criterion to define meals unless otherwise stated, but the data in Tables 8 and 9 are shown with shorter criteria as well. Where the use of a different criterion would change the results, data are described using more than one criterion. It should be recognized that, until a physiological or psychological basis is established for selecting a particular criterion, when the results vary with the criterion used, they should be interpreted with caution.

Meal Pattern Before Implantation of Electrodes

All rats ate distinct meals. There were no significant differences in either total intake or any of the meal parameters between the VMH-implanted and sham-operated groups before surgery (see Table 5). However, the size of meals and the number per day varied considerably between individual animals. The daily number of meals varied from a mean of 8.0 (R=7.0-9.0) in the animal taking the fewest meals (DD 8) to 13.7 (R=13.0-14.0) in the animal consuming the greatest number of meals (DD 9). Mean daily meal size varied from 1.16 (R=1.08-1.22) g per day in the animal taking the smallest meals (DD 9) to 2.07 (R=2.01-2.20) g per day in the animal taking the largest meals (DD 8). The animal which consumed the largest meals (DD 8) ate the fewest meals per day (see Table 5). The sizes and frequencies in the remaining animals were so close to each other that no other relationship could be seen between the size and frequencies (see Table 5).

Effects of Implantation of Electrodes or Sham Electrodes

As a result of the implantation of the electrodes, there was a significant increase in food intake (U=1, p=.029 n_1=4, n_2=4, Mann Whitney U test, two-tailed, Mendenhall, 1971) of 26.2 (R-18.6-38.3) percent (see Table 5). This increase, however, was not accompanied by any one specific change in meal parameters (see Table 5). In three of the VMH-implanted rats (DD 3, 7, 9) there was an increase in meal sizes of 7.8 to 31.0 percent. In 2 of these animals (DD 3, 9) there was also an increase in meal number of 10.0 and 14.5 percent while in the other animal (DD 7) the number of meals decreased by a small

Table 5. Intakes and Meal Parameters of Individual Animals Before and Following Implantation of Electrodes with a 40-Minute Criterion

Animal	Intake		Meal Size (g)			Meal Number		
	Total(g)	Per Cent Light Phase	Total	Light Phase	Dark Phase	Total	Light Phase	Dark Phase
			Before VMH-Implant					
DD3	19.9	24.2	1.66	1.20	1.88	12.0	4.0	8.0
DD7	14.1	29.2	1.28	0.91	1.54	11.0	4.5	6.5
DD8	16.6	20.0	2.07	1.71	2.33	8.0	3.3	4.7
DD9	15.8	38.3	1.16	0.96	1.32	13.7	6.3	7.3
Mean	16.6	30.4	1.54	1.19	1.77	11.2	4.5	6.6
			Before Sham-Implant					
DD4	19.0	31.1	1.50	1.11	1.79	12.7	5.3	7.3
DD5	16.7	31.5	1.39	1.12	1.56	12.0	4.7	7.3
Mean	17.9	31.3	1.45	1.12	1.68	12.4	5.0	7.3
U[1]	6	6	4	4	4	6	6	5
p[1]	>.600	>.600	.600	.600	.600	>.600	>.600	>.600
			Following VMH-Implant/Before Lesion					
DD3	23.6	30.8	1.79	1.39	2.04	13.2	5.0	8.2
DD7	19.5	33.3	1.53	1.35	1.63	12.8	4.8	8.0
DD8	20.4	26.3	1.79	1.44	1.93	11.4	3.4	8.0
DD9	20.0	32.6	1.52	1.14	1.81	13.2	5.0	7.6
Mean	20.9	30.8	1.66	1.33	1.85	12.7	4.7	8.0
U[1]	1	6	4	5	6	7	6	4
p[2]	.029	.343	.171	.243	.343	.443	.343	.171
			Following Sham-Implant/Before Anesthetization					
DD4	20.0	28.6	1.72	1.51	1.83	11.6	4.0	7.6
DD5	19.7	19.4	1.69	1.09	1.90	11.6	3.0	8.6
Mean	19.9	24.0	1.71	1.30	1.87	11.6	3.5	8.1
U[3]	4	1	6	4	4	2	1	5
p[3]	.600	.133	>.600	.600	.600	.267	.133	>.600

1. U and p are the values of the U (values of the Mann Whitney U Test (two-tailed, Mendenhall, 1971), and the probability that the intakes and meal parameters of implanted and sham-implant groups are the same before surgery.
2. U and p are the values of the Mann Whitney U Test (two-tailed, Mendenhall, 1971), and the probability that the intakes and meal parameters of the VMH-implanted group are the same before and following surgery.
3. U and p are the values of the Mann Whitney U Test (two-tailed, Mendenhall, 1971), and the probability that the intakes and meal parameters of the VMH-implanted and sham-implanted groups are the same following surgery.

amount (3.6 percent). The fourth implanted animal (DD 8) decreased its meal size by 13.5 percent and increased its meal number by 42.5 percent (see Table 5). This was also the animal which became least obese after lesions.

Both intake and meal parameters also increased in sham-implanted rats. Intake increased by 4.2 to 18.0 percent (see Table 5). Meal size increased by 14.7 to 21.6 percent and the frequency of meals decreased slightly (3.3 percent). The implantation of the electrodes had no apparent effect on the light-dark distribution of intake in either VMH-implanted or sham-operated rats (see Table 5).

Initial Effects of the Lesion

Within minutes (3-21) all lesioned rats began feeding. In the rats which subsequently became obese, in the first 3-4 hours following the lesion there were many (13-20) small (mean size 0.64 g, range = 0.51 -0.71 g) individual bouts of feeding each lasting an average of 3.9 (R=3.3-4.6) minutes. These small bouts were interspersed by a few (2-3) larger bouts whose size equaled or exceeded the mean pre-lesion meal size (using a 40-minute criterion). The individual bouts were separated by periods of no feeding averaging 7.9 (R=5.3-9.6) minutes. Although there were a few (2-3) longer pauses lasting as long as 32 minutes, in some rats there were no pauses of more than 40 minutes during the first 3-4 hours following the lesion. During this period following the animals consumed a mean of 56.2 (R=38.2-79.0) percent of their mean pre-lesion 24-hour intake.

The behavior described above was followed by a distinct period of

no eating in each rat which was at least one-third longer (41.7-82.3 minutes) than any of the pauses between feedings which occurred immediately following the lesion.

The animal which attained moderate obesity (DD 8) in contrast to the other lesioned rats immediately following the lesion ate continuously for approximately 20 minutes and consumed an amount of food which was approximately twice the size of the mean pre-lesioned meal. After 38 minutes another feeding bout of approximately the same size occurred. This bout was preceded briefly (3 minutes) by two small bouts (0.25 and 0.48 g). This second bout was followed by a much longer period of no eating (67 minutes, see Figure 7, line 2).

In summary, the initial effect of the lesion was to increase meal size using any reasonable criterion for separating meals. The effect of 1-, 5-, 20-, and 40-minute criteria upon the meal pattern profile during the immediate post lesion period is shown in Figures 4 through 7. The use of different criteria for defining intermeal intervals in the analysis of meal pattern data for this immediate post lesion period resulted in different descriptions of the behavior. If a 1-minute criterion was used, the feeding during this period could be described as consisting of many small individual bouts separated by many short pauses. If any other criteria from 5 to 32 minutes in length were used, the period following the lesion could be described as consisting of one or more bouts of sustained feeding larger than bouts which occurred before the lesion accompanied by many smaller shorter bursts of feeding. The frequency, size, and duration of the larger bouts and the frequency of the small bursts would depend upon

Figure 4. Meal pattern profiles with 1-, 5-, 20-, and 40-minute criteria for the first post-lesion day (lines B) and the day immediately preceding lesion (lines A) for obese rat DD.7. Each line shows the temporal pattern of feeding for the entire 24-hour period. Elevation of the trace above baseline indicates feeding. Amount taken is indicated by the height of the trace above baseline (see scale, lower left). Duration of the meal is indicated by the length of time (across horizontal axis) trace remains elevated. The trace remained elevated and continued moving upward as long as the animal continued to eat with pauses of less than 1 min and did not reset to baseline unless there was no additional eating for the period specified by the criterion. The arrows above lines B indicate the exact time the VMH lesion was made. The arrows beneath each line indicate the time when food and water measurements were made.

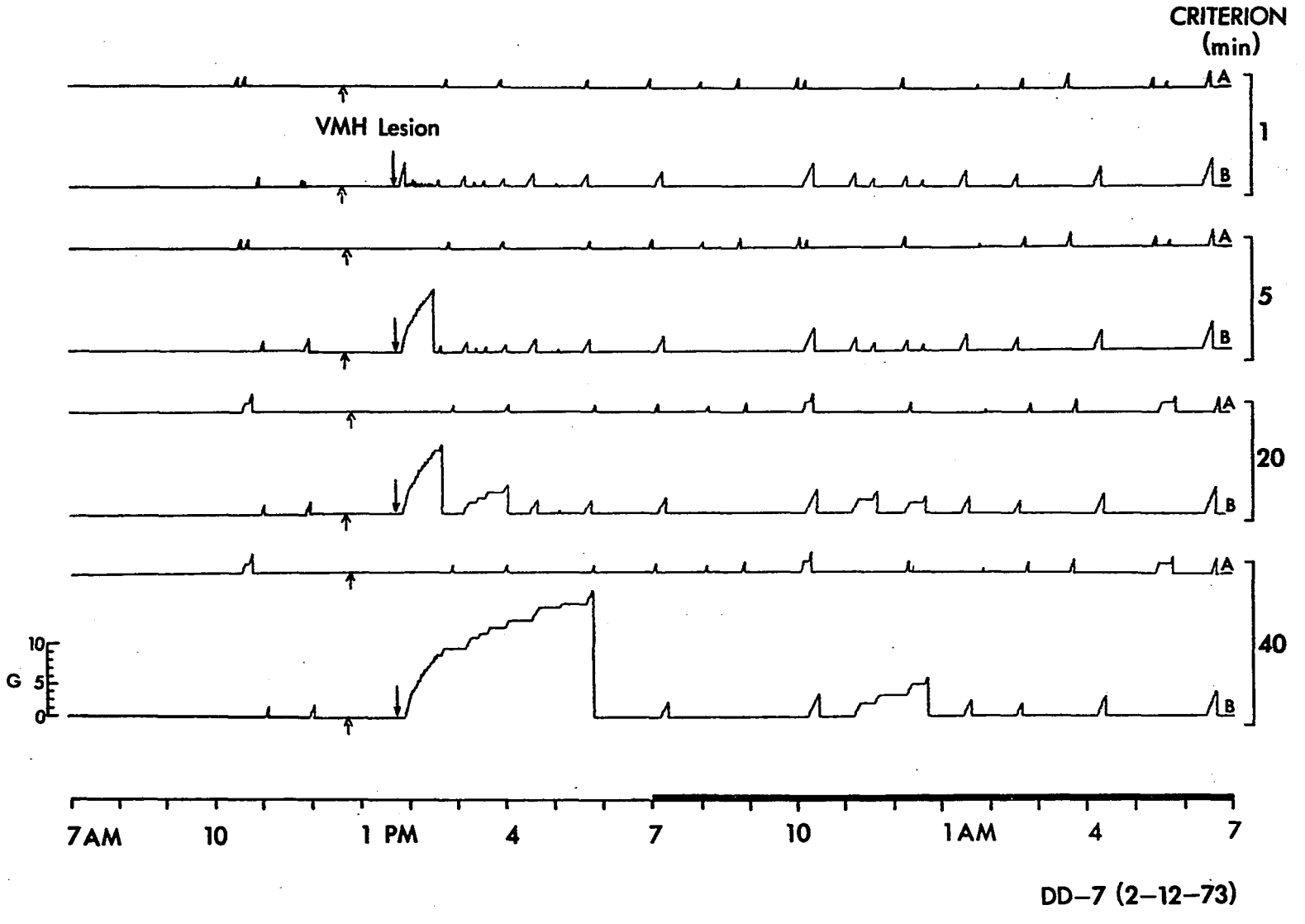

CRITERION
(min)
VMH Lesion
A
B
1
5
20
40
G
10
5
0
7AM
10
1 PM
4
7
10
1AM
4
7
DD–7 (2–12–73)

Fig. 5. Meal patterns profiles with 1-, 5-, 20-, and 40-minute criteria for the first post-lesion day (lines B) and the day immediately preceding lesion (lines A) for obese rat, DD 3. For detailed explanation see Figure 4.

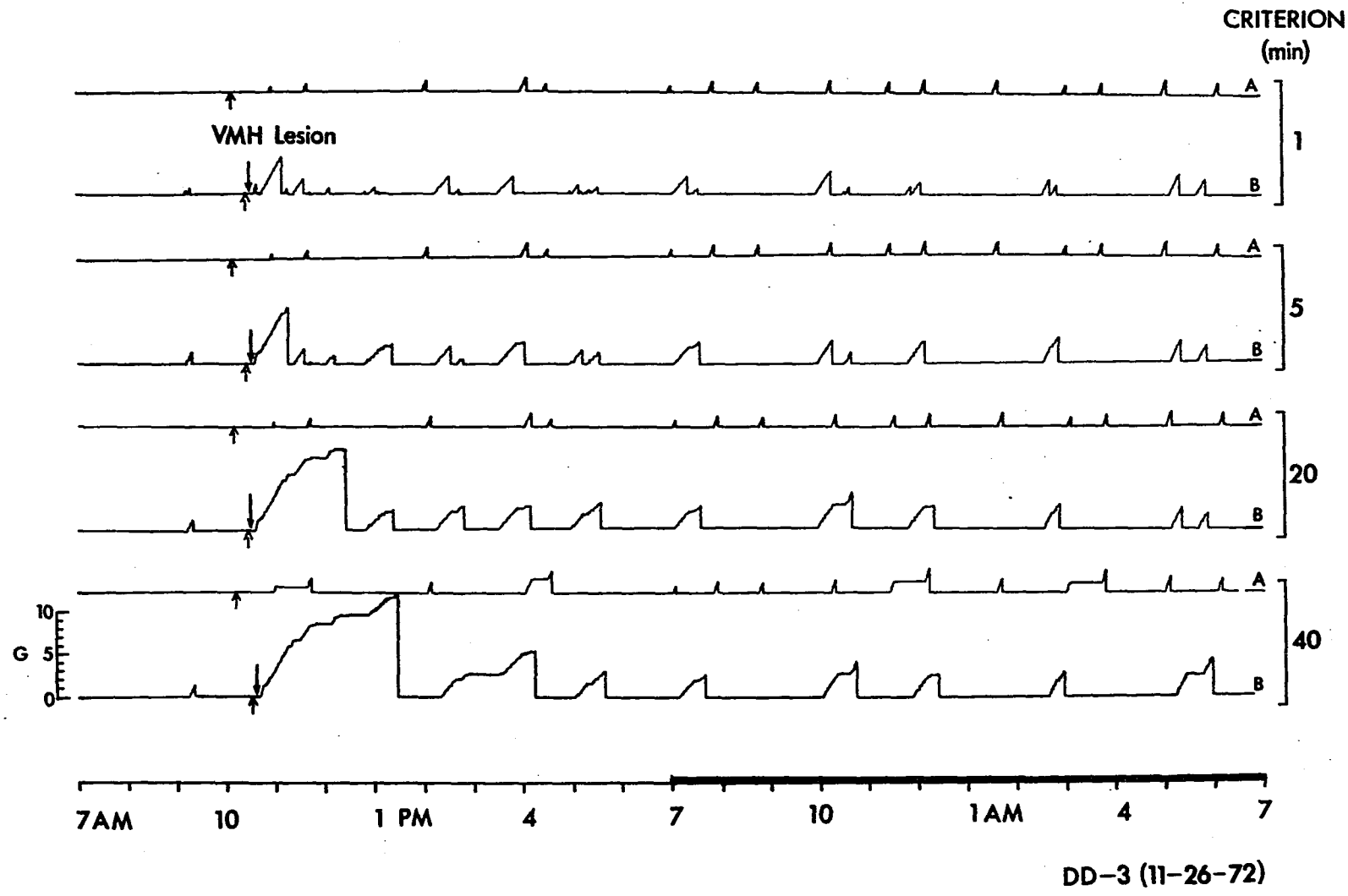

CRITERION
(min)
VMH Lesion
A
B
1
5
20
40
G
10
5
0
7AM
10
1 PM
4
7
10
1AM
4
7
DD–3 (11–26–72)

Figure 6. Meal pattern profiles with 1-, 5-, 20-, and 40-minute criteria for the first post lesion day (lines B) and the day immediately preceding (lines A) for obese rat DD 7. For detailed explanation see Figure 4.

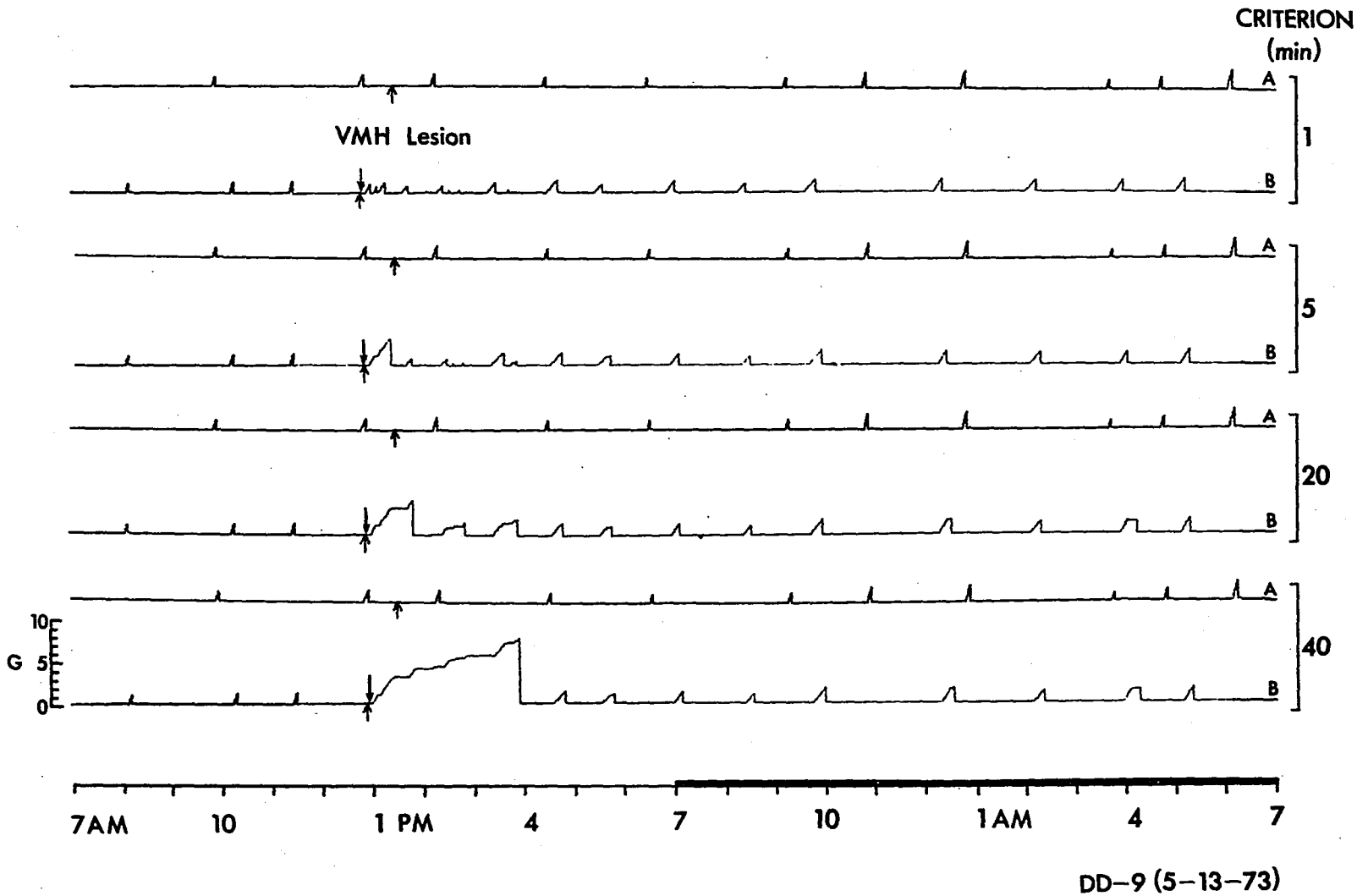
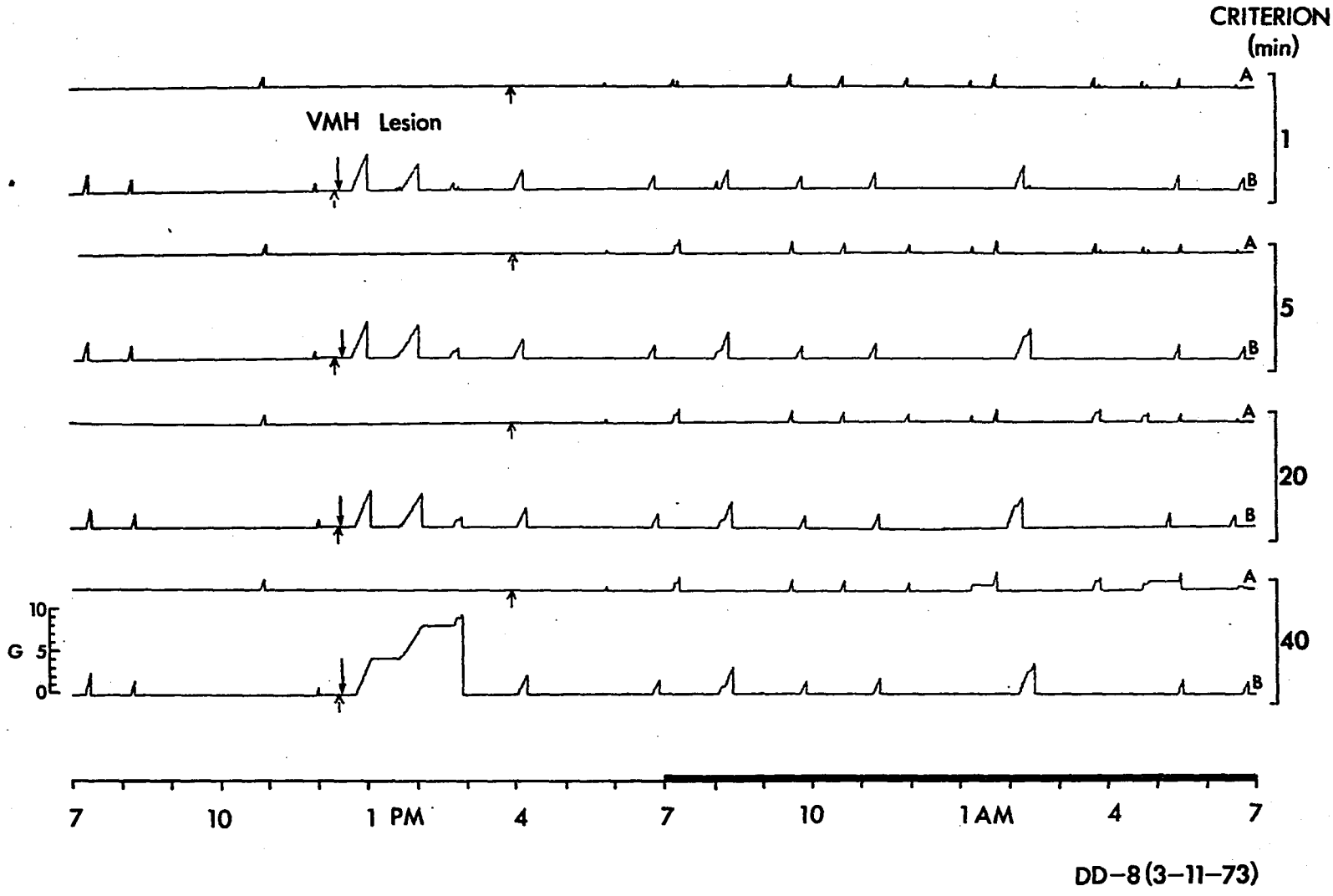

CRITERION
(min)
VMH Lesion
A
B
1
5
20
40
G
10
5
0
7AM
10
1 PM
4
7
10
1AM
4
7
DD-9 (5-13-73)

Figure 7. Meal pattern profiles with 1-, 5-, 20-, and 40-minute criteria for the first post lesion day (lines B) and the day immediately preceding (lines A) for moderately obese rat, DD 8. For detailed explanation see Figure 4.


CRITERION
(min)
VMH Lesion
A
B
1
5
20
40
G
10
5
0
7
10
1 PM
4
7
10
1 AM
4
7
DD-8 (3-11-73)

the particular criterion chosen. If any criteria larger than 32 minutes were used, the period following the lesion could be described as one discrete bout of feeding several times larger than any previous bout and lasting as long as 3 hours.

Dynamic Phase

The dynamic phase was characterized by rates of body weight gain several times greater than normal (see Table 6), extreme hyperphagia accompanied throughout by equal intakes during the light and dark periods (see Table 7), a criterion-independent increase of the mean daily meal size (see Table 8), and a criterion-dependent increase in mean daily meal number. The mean daily meal size increased for 5-6 days, leveled off for 5 days, and then declined slightly. While meal size was declining the mean daily number of meals increased by a small amount (see Table 8). The quantitative effects of the lesion on meal size and frequency were found to be strongly dependent upon the criterion chosen to separate meals. If a 5-minute criterion was used, the increase in meal size was much less than the increase in meal number, while if a 40-minute criterion was chosen, the reverse was true, i.e., meal size increased much more than meal number. A 20-minute criterion favors an increase in meal size with a much smaller increase in meal number (see Table 8).

The rates of weight gain in all lesioned rats classified as obese exceeded the pre-lesion rate of gain during the first 15 days following the lesion (see Table 6). The rate of body weight gain in the moderately obese rat during the first 3 days following the lesion was excessive when compared with the pre-lesion rate of gain, but not as great as the rate of gain seen in the obese rats

Table 6. Rates of Body Weight Gain, g/day

Animal	Pre-Lesion (5 days)	POST LESION DAYS 1-3	4-10	11-15	16-20	20-30	30-60
				Obese			
DD 3	2.8	21.0	10.7	8.6	0.8	1.3	2.0
DD 7	0.6	22.3	10.4	11.2	9.0	7.2	2.2
DD 9	1.2	22.0	7.2	4.0	3.8	-0.4	1.3
Mean	1.5	21.8	9.4	8.9	4.5	2.7	1.8
				Moderately Obese			
DD 8	0.2	15.0	3.0	3.2	2.2	0.6	1.3
				Sham Lesion			
DD 4	2.0	-0.3	-0.1	0.4			1.2
DD 5	1.2	-1.7	1.9				0.5
Mean	1.6	-1.0	0.9				0.9

Table 7. Mean Daily Intake, in Grams

	Both Phases	Light Phase	Dark Phase	U*	p*
		Pre-Lesion			
Obese (N=3)	21.0 (19.5-23.6)	6.8 (6.5-7.3)	14.3 (13.0-16.3)		†
Moderately Obese (N=1)	20.4	5.4	15.0		
Sham Lesion (N=2)	19.9 (19.7-20.0)	4.8 (3.8-5.7)	15.1 (14.3-15.8)		
		1-3 Days Post Lesion			
Obese (N=3)	37.6 (30.1-41.8)	21.4 (19.1-23.3)	16.2 (11.1-19.2)		†
U** p**	ǂ	ǂ	ǂ		
Moderately Obese (N-1)	31.9	16.9	15.0		
Sham Lesion (N=2)	19.0 (18.9-19.0)	5.5 (3.7-7.3)	13.4 (11.7-15.14)		
		4-10 Days Post Lesion			
Obese (n=3)	47.2 (35.2-54.7)	23.7 (16.5-28.4)	23.5 (18.7-26.3)	4	.50
U** p**	ǂ	ǂ	ǂ		
Moderately Obese (N=1)	25.4	12.0	13.4		
Sham Lesion (N=2)	19.1 (17.0-21.2)	5.8 (5.7-5.9)	13.3 (11.1-15.5)		
		11-15 Days Post Lesion			
Obese (N=3)	49.4 (33.6-59.7)	24.3 (15.4-30.0)	25.1 (18.1-29.8)	4	.50
U** p**	ǂ	ǂ	ǂ		
Moderately Obese (N=1)	25.0	11.1	13.9		
Sham Lesion	16.9	5.2	11.7		

Table 7 Continued. Mean Daily Intake, in Grams

	Both Phases	Light Phase	Dark Phase	U*	p*
		16-20 Days Post Lesion			
Obese (N=3)	45.6 (32.1-57.2)	22.4 (14.7-28.1)	23.3 (17.4-29.1)	4	.50
U_2^2	-	-	-		
p^2	ǂ	ǂ	ǂ		
Moderately Obese (N=1)	23.6	12.1	11.4		
		65-75 Days Post Lesion			
Obese (N=3)	26.0 (24.5-28.3)	13.1 (11.4-15.3)	12.9 (11.9-13.9)	4	.50
U_2^2	-	-	2		
p^2	ǂ	ǂ	.10		
Moderately Obese (N=1)	21.2	10.4	10.9		
Sham Lesion (N=2)	21.5 (19.8-23.1)	6.7 (4.9-8.6)	14.7 (14.5-14.9)		

* - U and p are the values of the Mann Whitney U Test (two-tailed, Mendenhall, 1971) and the probability that the intakes during the light and dark phases are the same.

** - U and p are the values of the Mann Whitney U Test (two-tailed, Mendenhall, 1971) and the probability that the intakes are the same before and following the lesion.

† - no overlap in range of light phase and dark phase intakes.

ǂ - no overlap in range of pre-lesion and post lesion intakes.

Table 8. Mean and Range of Daily Mean Meal Size and Number of Meals of Lesioned Rats

	Meal Size (g)			Meal Number		
Criterion (min)	5	20	40	5	20	40
			Obese (N=3)			
Condition						
Pre-Implant	1.17 (1.03-1.35)	1.20 (1.03-1.39)	1.37 (1.16-1.66)	14.2 (12.5-15.3)	13.9 (12.0-15.3)	12.2 (11.0-13.7)
Pre-Lesion	1.39 (1.30-1.39)	1.45 (1.42-1.48)	1.62 (1.52-1.79)	15.6 (14.8-17.0)	14.6 (13.2-16.6)	13.0 (12.6-13.2)
Post Lesion 1-3	1.69 (1.20-2.08)	2.29 (1.67-2.85)	2.87 (2.17-3.40)	22.7 (19.7-25.0)	17.4 (14.3-20.0)	13.3 (12.0-14.0)
Post Lesion 4-10	2.07 (1.16-3.51)	2.55 (1.45-4.41)	3.38 (2.17-5.47)	21.3 (15.6-30.4)	17.4 (12.4-24.6)	12.4 (10.0-14.4)
Post Lesion 11-15	2.27 (1.17-3.65)	2.82 (1.46-4.08)	3.47 (2.51-5.47)	24.2 (16.3-28.8)	19.0 (15.0-23.0)	14.8 (13.7-15.8)
Post Lesion 16-20	2.29 (1.19-3.66)	2.69 (1.34-4.08)	3.14 (1.88-4.47)	22.3 (16.3-27.0)	19.0 (15.0-24.0)	15.7 (13.7-17.8)
Post Lesion 65-75	1.75 (0.90-2.94)	1.98 (1.21-3.10)	2.35 (1.90-3.29)	18.6 (9.6-27.1)	15.5 (9.1-20.8)	11.9 (8.6-13.7)
			Moderately Obese (N=1)			
Pre-Implant						
Pre-Lesion	1.41	1.54	1.79	14.4	13.2	11.6
Post Lesion 1-3	2.23	2.23	2.39	14.3	14.3	13.3
Post Lesion 6-10						
Post Lesion 11-15	3.13	3.57	3.57	8.0	7.0	7.0
Post Lesion 65-75	2.90	3.08	3.13	7.3	6.9	6.8

(see Table 6). The rate of gain declined more rapidly in the moderately obese rat than in the obese animals.

Food intake gradually increased, and maximum intake was reached in individual animals 9 to 15 days following the lesion and was 92.9 to 219.9 percent greater than before the lesion. In the moderately obese rat intake did not gradually increase, but maximum intake occurred on the first day following the lesion and was 67.2 percent greater than before the lesion. In this rat on the 10th day following the lesion intake was only 13.6 percent greater than before the lesion.

In all lesioned rats both obese and moderately obese the normal predominately nocturnal occurrence of feeding disappeared and intake was evenly divided between the light and dark phases during all post lesion periods.

Mean daily meal size was immediately increased by the lesion in both obese and moderately obese rats. In 2 of the 3 lesioned obese rats mean daily meal size gradually increased and reached a maximum on the 5th and 6th days following the lesion respectively. Mean daily meal sizes at this time were 90.8 to 387.2 percent greater than before the lesion. In the other lesioned rat (DD 9) maximum mean daily meal size occurred on the first day following the lesion, and this was the result of the large initial meal. In all obese animals the extended period when mean daily meal size was most enlarged occurred between days 4-10 post lesion when it was 65.1 to 252.9 percent greater than pre-lesion (see Table 8). Following this period in all obese rats, mean

daily meal sizes began to slowly decline and between the 16th and 20th days post lesion ranged from 21.2 to 167.7 percent greater than before the lesion.

Meal number remained unchanged from pre-lesion during the first 10 days following the lesion using the 40-minute criterion (see Table 8). From the 11th to 15th days and from the 16th to the 20th days the mean daily number of meals increased in all animals (see Table 8). These increases ranged from 8.7 to 25.0 percent for the 11th-15th days and 11.1 to 34.8 percent for the 16th to 20th days. Using smaller criteria meal number increased earlier in the post lesion period and to a greater extent (see Table 8, 5-minute criterion).

The changes in meal parameters which occurred in the moderately obese rat were not the same as occurred in the obese animal. In this rat (DD8) mean daily meal sizes steadily increased and the mean daily number of meals steadily decreased in the 20 days following the lesion (see Table 8).

Static Phase

The static phase was characterized by normal rates of weight gain (see Table 6), food intake appreciably less than immediately following the lesion but significantly greater than before it (see Table 7), and a continued disruption in the nychthemeral distribution of feeding (see Table 7). Mean daily meal size was greater than before the lesion but the daily number of meals at first was elevated, then unchanged, or reduced as the criterion increased from 5 to 40 minutes (see Table 8).

Mean daily food intake in the obese lesioned rats ranged from 7.2 to 45.1 percent greater than before the lesion. For the three obese animals there was a perfect positive rank order correlation between food intake and mean daily meal size and a perfect negative correlation between mean food intake and mean daily number using any criterion between 5 and 40 minutes in length (see Table10). Mean daily meal size was 15.4 to 109.5 percent greater than before the lesion in two rats (DD 3, DD 7) and decreased by 16.0 percent in the other rat (DD 9) using the 40-minute criterion. Two of the rats ate approximately the same number of meals as before the lesion, but in the third rat, mean daily meal number was decreased by 38.5 percent. The animal whose meal number was decreased was the animal which was eating the biggest meals. These mean parameters were not related to meal parameters of these animals before the lesion (cf. Tables 5, 9).

Intake in the moderately obese rat was the same as before the lesion. Both the distribution of intake (see Table 7) and meal parameters were altered (see Table 8). Meal sizes in this rat were exactly double the pre-lesion size, and the number of meals per day was approximately half as large as before the lesion (see Table 8).

Sham-Lesioned Animals

The sham lesion had little effect on food intake (see Table 7), its nychthemeral periodicity, and meal parameters (see Table 9). Throughout the course of the experiment both food intake and meal parameters in these animals showed little change (see Figure 3). This demonstrates that both meal parameters and the distribution of intermeal intervals

Table 9. Mean and Range of Mean Daily Meal Size and Number of Meals of Sham-Lesioned Rats

	Meal Size (g)			Meal Number		
Criterion (min)	5	20	40	5	20	40
Condition						
Pre-Implant	1.30 (1.27-1.32)	1.34 (1.33-1.35)	1.45 (1.39-1.50)	13.9 (12.7-15.0)	13.3 (12.3-14.3)	12.4 (12.0-12.7)
Pre-Sham Lesion	1.31 (1.07-1.56)	1.37 (1.21-1.56)	1.84 (1.69-1.72)	15.6 (12.8-18.4)	14.5 (12.8-16.2)	11.6 (11.6-11.6)
Post-Sham Lesion Days 1 - 3	1.54 (1.45-1.63)	1.61 (1.53-1.68)	1.78 (1.68-1.88)	12.3 (11.7-13.0)	11.8 (11.3-12.3)	10.6 (10.0-11.3)
Post-Sham Lesion Days 4 - 10	1.70 (1.585-1.816)	1.73 (1.59-1.82)	1.85 (1.70-1.99)	11.2 (10.7-11.7)	11.0 (10.7-11.3)	10.4 (10.0-10.7)
Post-Sham Lesion Days 11 - 15	1.96	1.96	2.01	8.6	8.6	8.4
Post-Sham Lesion Days 65 - 75	1.56 (1.50-1.61)	1.74 (1.72-1.75)	1.86 (1.83-1.92)	13.9 (12.3-15.4)	12.5 (11.3-13.6)	11.5 (10.8-12.2)

Table 10. Relationship of Food Intake to Meal Parameters for Obese VMH-Lesioned Rats in Static Phase (days 65-75 post lesions)

Rat	Mean Daily Food Intake (g)	Mean Daily Meal Size (g)		Mean Daily Number of Meals	
		5-min. crit.	40-min crit.	5-min. crit.	40-min. crit.
DD7	28.3	2.94	3.16	9.6	8.6
DD3	25.3	1.40	2.02	18.1	13.3
DD9	24.3	0.90	1.86	27.1	13.7

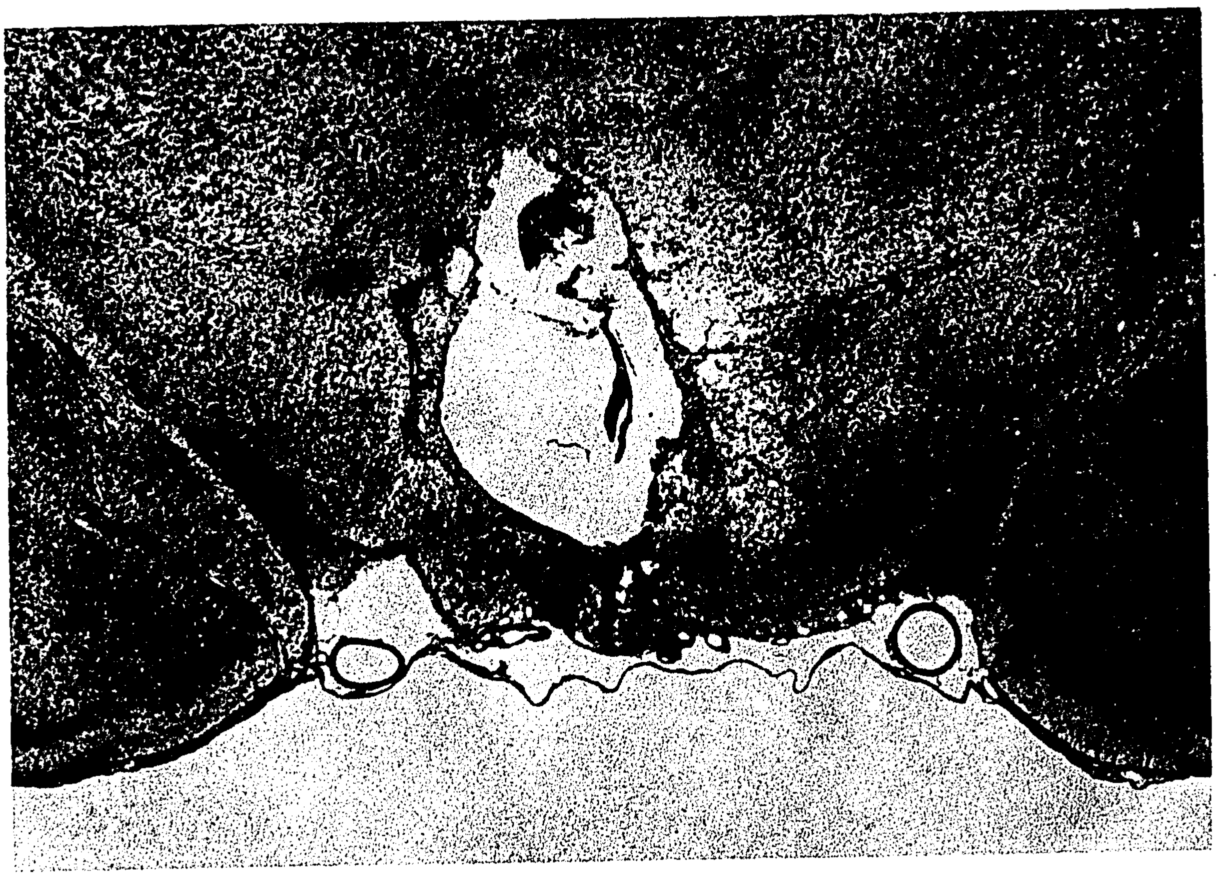
remain relatively stable in normal rats over a period equal to the time course of this experiment.

Histology

The most hyperphagic and obese rat (DD 7) (see Figure 8) had an extremely large lesion centered in the anterior pole of the ventromedial nucleus of the hypothalamus approximately at the level of plate #5150 (Konig & Klippel, 1963). The lesion at this level destroyed all or most of the ventromedial nucleus, the dorsomedial nucleus, the third ventricle, fornix, and mammilothalamic tract extending to the level of the median forebrain bundle just in front of the paraventricular nucleus (Konig & Klippel, 1963, plate #5630). The lesion also destroyed the anterior hypothalamic nucleus and extended posterially to the level of Konig and Klippel (1963) plate #3290. Obese rat (DD 3) had a large lesion centered in the ventromedial nucleus of the hypothalamus (Konig & Klippel, 1963, plate #4380) which extended throughout the ventromedial hypothalamus. DD 9 had a smaller lesion centered at the anterior pole of the subthalamic nucleus. The central portion of the ventromedial nucleus was intact, but there was damage to the dorsomedial nucleus.

The moderately obese animal, DD 8 had a small lesion centered toward the posterior section of the ventromedial nucleus (Konig & Klippel, 1963, plate #4620). The lesion appeared to spare most of the ventromedial nucleus on one side, but damaged the third ventrical and portions of the dorsomedial nucleus. The lesion extended anteriorally to the anterior pole of the ventromedial hypothalamus (Konig & Klippel, 1963, plate #4890) and posteriorally to the level of plate #3290 (Konig & Klippel, 1963).

Figure 8. Photomicrograph of coronal section at the point of maximal extent of the lesion of obese rat, DD 7.



The results of this experiment support a recent report which demonstrates that maximum obesity occurs with lesions that overflow the ventromedial nucleus (Gold, 1973).

Discussion

The results of this experiment extend the major findings of Becker and Kissileff (1974) to a solid diet. These findings were as follows.

1) An initial large meal beginning within minutes of the VMH lesion which was larger than any previous meal.
2) A series of sequential changes in meal parameters following the VMH lesion and accompanying the hyperphagia beginning with an increase in meal size for several days followed by a leveling off of meal size for several more days, and then a gradual decline in meal size. When meal size declined, hyperphagia was maintained by an increase in the daily number of meals. When the animals became obese food intake returned to the pre-lesion level as a result of a decrease in meal frequency.
3) During the whole time the above changes were taking place, the normal nychthemeral pattern of predominately nocturnal feeding was replaced by equal intakes during the light and dark phases.

The first two of these three findings are clearly shown when a 40-minute criterion is used to define intermeal intervals. With shorter criteria, the same conclusions can be reached but they are

less impressive. For example, meal size increased from 1.17 g before the lesion to 2.29 g, 16-20 days after the lesion using a 5-minute criterion, but increased from 1.37 g to 3.14 g when a 40-minute criterion is used. Thus a larger criterion favors increased intake by an increase in meal size. Conversely, meal number increases less with a larger criterion than with a smaller one. For example, meal number increased from 14.2 before the lesion to 18.6, 16-20 days after the lesion, with a 5-minute criterion, but only from 12.2 to 15.7 with a 40-minute criterion.

These results underscore the point made by Kissileff (1970) that the criterion chosen to separate meals can be an important determining factor in the quantitative description of meal-taking patterns and possibly in the interpretation placed upon them. They also illustrate the importance of drawing conclusions about meal patterns in general only when more than one diet is utilzed. The main theme of the present experiment is that when the criterion for meal termination is appropriately chosen, on the basis of the distribution of intermeal intervals, and in the present case, 40-min with a dry diet, the description of the meal pattern of VMH-lesioned rats becomes identical to that for VMH-lesioned rats eating a liquid diet. The early effects of the lesion in both cases produce an animal which overeats exclusively by increasing the size of its meals. The differences in pattern seen with the two diets can therefore be attributed to the influence of the diet on meal patterns. Solid diets tend to promote more short pauses in meals than do liquid diets. Therefore a larger criterion is more appropriate for separating meals. The effect of the VMH-lesion, on

meal taking then becomes a unitary phenomenon. On both diets meal size is the exclusive means of expression of hyperphagia.

There are several possible reasons that VMH-lesioned rats pause more frequently when eating solid diets than when eating liquid diets. First, since VMH lesions often produce finickiness, it is possible that VMH-lesioned rats find solid diets less palatable than liquid diets. A recent experiment (Sclafani, 1974) has shown that when VMH-lesioned rats are fed a sweetened condensed milk adultered with quinine they reduce meal sizes and increase meal frequency (data analyzed with a 10-minute criterion for defining intermeal intervals), and it is possible that a similar effect occurs with solid diets. Second, it is possible that it might be difficult for an animal to consume a large meal rapidly with a solid diet because a solid diet may produce both dryness and cause the animal to interrupt the meal for draughts of water. If this were the case, then an effect similar to the one observed here should occur under other conditions (e.g., ovariectomy, see Kenney and Mook, 1974) where meal size is enlarged. The data from other published experiments has not been examined in sufficient detail to determine if this occurs. Furthermore, in the present experiment no attempt was made to determine whether drinking occurred, during the short interruptions (less than 10 minutes) between the bouts of feeding. Examination of the meal pattern of the lactating rat (see experiment 4) does not support this possibility as the exclusive explanation. Lactating rats eat dry foods in larger meals than VMH-lesioned rats with an increase in the frequency of short interruptions in feeding.

However the number of short interruptions (less than 10 min) increased much less (by about 1-2 meal per day) in the lactating rat than it did in the VMH lesioned rat (increased by 6-13 per day; see figure 2).

These results are similar to those of Teitelbaum and Campbell (1958) who used an independent group design. There was, however, one difference. In the present experiment with a 5-minute criterion there was a significant increase in meal frequency. Teitelbaum and Campbell (1958) reported an increase in meal frequency which was not significant, and their increase in meal size was also the exclusive means by which hyperphagia was expressed. The novel information in the present experiment and in that of Becker and Kissileff (1974) is the immediacy with which the phenomenon appears when the animal is not surgically manipulated and anesthetized with barbiturates when the lesion is made.

These initial effects of the lesion differed from the earlier report of Balagura and Devenport (1970). Balagura and Devenport reported that their animals ate persistently for several hours following the lesion (the representative female rat whose data they show ate persistently for approximately four and one-half hours) without a pause of at least 20 minutes. In the present investigation when the data was analyzed with the same criterion no similar effect occurred (cf. Figures 4, 5, 6, 7, 20-minute criterion with Balagura and Devenport, 1970, Figure 2), and the data more closely resembled the findings of Becker and Kissileff (1974). However, the initial response to the lesion with a solid diet observed in this experiment did differ in certain respects from the initial response that occurred with a liquid

diet. Following the lesion the number of small pauses in eating was considerably greater with solid diets than before the lesion, but not different with liquid diets (Becker & Kissileff, 1974); and as a result the meals lasted longer with the solid diet than with the liquid diet. These data show therefore that the differences between our previous report (Becker & Kissileff, 1974) and the findings of Balaguara and Devenport (1970) are not due to differences in diet. The possibility that they are due to differences in the metal used to make the lesion or the coincidence of surgery and barbiturate anesthesia with the lesion still need to be explored.

The last observation of importance is the separation of effects of lesions on meal parameters and total daily food intake. DD 8 is an important example of this phenomenon, since her meal size increased and remained elevated, while after weight gain, meal number was reduced and total food intake remained at its basal level. This finding reinforces the suggestion made by Becker and Kissileff (1974) that small lesions which influence only a single meal parameter will be more important in unraveling the neural mechanism controlling feeding than global lesions which may simultaneously disrupt several systems at once.

On the other hand, when the three obese animals were in the static phase there was a correlation between food intake and meal size which was not present before lesioning. This suggests that even though the animals are now at their new "set point," there are still permanent alterations in meal parameters. It may be that either set points for body weight are not related to meal parameters or alterations in body weight are not related

to changes in set point which would imply that all perturbations needed to reach the new set point return to normal when it is reached. Another possibility discussed later (p. 205) is that the set point for body weight is not impaired in the VMH-lesioned rat but that the feedback signals to a hypothetical comparator are blunted by the lesion (Hirsch, 1972) and as a result body weight stabilized at an elevated level.

EXPERIMENT 2. Meal Patterns of the Genetically Obese Rat

The genetically obese "fatty" rat identified by Zucker and Zucker (1961) is hyperphagic (Zucker, 1965; Barry & Bray, 1969; Bray & York, 1972), gains weight progressively throughout life (Bray & York, 1971b), and becomes tremendously obese. In addition, the genetically obese rat is hyperinsulinemic (see Bray & York, 1971a for review), infertile due to a lack of estrous behavior (Bray, 1970), deficient in thyroid stimulating hormone secretion (York, Hershman, Utiger,& Bray, 1972), and has an altered water intake indicative of a primary polydipsia (York & Bray, 1971). Taken together these deficits suggest some congenital hypothalamic dysfunction.

There is no direct evidence to suggest that the hyperphagia results from this hypothalamic disturbance. It is not known, however, what alterations in feeding behavior accompany the hyperphagia in the "fatty" rat. If these alterations resemble the changes which occur following VMH lesions, then this might be evidence that the hyperphagia in the "fatty" rat might at least partially result from a generalized hypothalamic disturbance since it is possible that the similar alterations in feeding behavior in genetically obese and VMH lesioned rats result from disruption in the same mechanism.

Method

Continuous records of meal-taking patterns were obtained in genetically obese and nonobese sibling controls.

Animals

Four male genetically obese "fatty" rats (fa/fa) and 4 male nonobese sibling controls (Fa /-) were used in the experiment. At the start of

the procedures the animals were 90-105 days old. The genetically obese rats weighed between 500 and 595 (mean 534) g while the sibling controls weighed between 320 and 410 (mean 361) g . During the course of the experiment the genetically obese rats gained a mean of 17 g (R=2-46) while the controls gained a mean of 18.3 g (R=13-25).

Apparatus

While feeding patterns were collected, the animals were housed individually (see apparatus: liquid diets) and fed a liquid milk diet (see diets). Meal patterns were recorded (see automatic recording of feeding patterns) and analyzed with computer programs (see computer programs).

Environment

Four of the individual living chambers in which meal pattern data were collected were placed in a ventilated box with white walls inside. The inside dimensions of this box were 76.2 x 76.2 x 76.2 cm. The source of illumination was a 6-watt fluorescent lamp suspended 66 cm above the floor of the living chambers. The ambient illumination at the chamber floor was 5-foot candles (measured with a Weston model 614 foot candle meter. A 12 h - 12 h light-dark cycle (light on 6 A.M., off 6 P.M., local time) was employed. The temperatures during the course of the experiments ranged from 21-28^{o} C.

Sequence of Procedures

Prior to this experiment, the animals were housed individually in mesh-bottom cages with ad libitum access to Purina laboratory chow pellets and water. Before meal pattern data was collected, all animals were placed on the liquid milk diet for 5 - 7 days. For the next consecutive

10 days, body weight to the nearest gm and water intake to the nearest ml were measured daily and meal patterns were recorded.

Criterion for Intermeal Intervals

The distribution of intervals between meals was used to determine the appropriate criterion for the separation of individual meals. It was found that using this method the least frequent intervals between meals using a 1-minute criterion initially were between 20 and 30 minutes in both the genetically obese and normal controls. Accordingly, a 20-minute criterion was used to interpret the results. In order to demonstrate that the use of this criterion did not bias the results, the data were also presented using 5- and 40-minute criteria to define intermeal intervals.

Results

The mean daily food intake of the genetically obese rats exceeded the intake of the controls by approximately 30 per cent (see Table 11). The increased intake in the "fatty" rats was accompanied by obvious alterations in the normal meal pattern (see figure 9 for meal pattern profiles of representative obese rat, Fat 2, and a representative sibling control, Slim 1). First, the normal pattern of predominantly nocturnal feeding was replaced by equal intakes during the light and dark phases (see Table 11), and therefore the meal patterns during the light and dark periods were indistinguishable. Second, the size of individual meals and hence the mean daily meal size in the genetically obese rats was more than double that of the controls (see Table 12).

Hyperphagia in the genetically obese rats was characterized by increased intake during the part of the day when the lights were on.

Figure 9. Meal pattern profiles of a representative genetically obese rat (Fat 2) and a sibling control rat (Slim 1). For a detailed description of the meal pattern profile, see Figure 1. The number in parentheses under the dates are the amounts of food consumed in ml on the date shown for which the meal profile is presented.

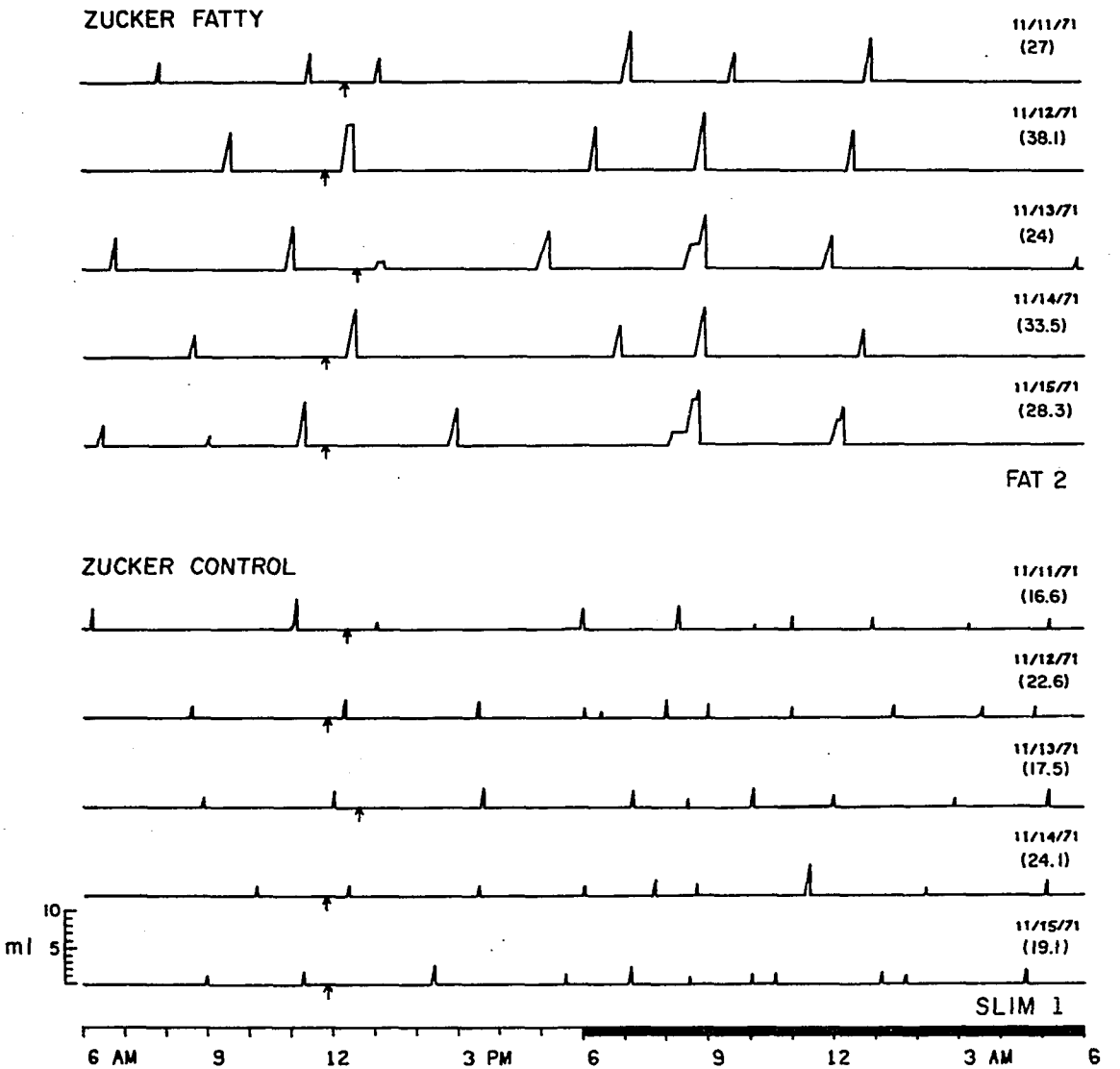

ZUCKER FATTY
11/11/71
(27)
11/12/71
(38.1)
11/13/71
(24)
11/14/71
(33.5)
11/15/71
(28.3)
FAT 2
ZUCKER CONTROL
11/11/71
(16.6)
11/12/71
(22.6)
11/13/71
(17.5)
11/14/71
(24.1)
11/15/71
(19.1)
10
ml 5
SLIM 1
6 AM
9
12
3 PM
6
9
12
3 AM
6

Table 11. Mean Daily Intake in Milliliters

Animal	Total Intake	Light Phase Intake	Dark Phase Intake	U[1]	p[1]
		Genetically Obese			
Fat -1	27.9 (20.4-36.3)	14.9 (8.0-22.9)	13.1 (10.5-19.5)		
Fat -2	29.9 (24.6-38.6)	14.3 (9.2-17.8)	15.6 (10.2-20.4)		
Fat -3	32.3 (23.6-41.0)	19.3 (9.4-25.4)	13.1 (6.3-19.5)		
Fat -4	28.1 (22.0-33.8)	14.0 (9.2-16.4)	14.0 (7.2-18.5)		
Mean	29.6	15.6	13.9	4	.171
		Litter-mate Control			
Slim -1	19.7 (15.7-22.6)	7.6 (4.7-11.7)	12.2 (8.1-14.4)		
Slim -2	21.7 (17.6-24.0)	7.2 (4.2-9.5)	14.5 (10.0-18.3)		
Slim -3	25.8 (20.3-29.5)	11.9 (8.2-15.4)	13.9 (10.0-21.6)		
Slim -4	24.1 (21.6-28.0)	9.9 (6.7-13.01)	14.2 (11.2-16.4)		
Mean	22.3	9.2	13.1		
U[2]	0	0	8		
p[2]	.014	.014	.557		

1. Columns U and p are the values of the Mann Whitney U Test (two-tailed) and probability that light and dark phase are the same.
2. Rows U and p are the values of the Mann Whitney U Test (two-tailed) that genetically obese and sibling

Table 12. Mean Daily Meal Size in Milliliters.

	Criterion (min.)	Both Phases	Light Phase	Dark Phase	U^1	p^1
Genetically Obese, N = 4	5.0	4.57 (3.95-6.06)	4.47 (3.54-6.67)	4.76 (4.22-5.23)	4	.171
	20.0	4.77 (4.01-6.32)	4.64 (3.69-6.68)	4.99 (4.39-5.87)	6	.343
	40.0	5.07 (4.32-6.46)	4.89 (4.10-6.93)	5.31 (4.39-5.87)	5	.243
Sibling Control, N = 4	5.0	1.97 (1.40-2.83)	2.02 (1.41-2.71)	1.97 (1.18-2.95)	5	.243
	20.0	2.16 (1.78-2.86)	2.15 (1.67-2.77)	2.18 (1.63-2.95)	7	.445
	40.0	2.40 (2.12-2.86)	2.26 (1.84-2.77)	2.53 (2.25-2.95)	4	.171
U^2		0	0	0		
p^2		.014	.014	.014		

1. Colums U and p are the values of the Mann Whitney U Test (two-tailed, Mendenhall, 1971) and probability that light and dark meal sizes are the same.
2. Rows U and p are the values of the Mann Whitney U Test (two-tailed, Mendenhall, 1971) and probability with a 20-minute criterion that the meal sizes of the genetically obese and sibling control are the same.

Table 13. Mean Daily Number of Meals

	Criterion (min.)	Both Phases	Light Phase	Dark Phase	U^1	p^1
Genetically Obese, N = 4	5.0	6.61 (5.33-7.30)	3.82 (2.89-5.0)	2.99 (2.5-3.70)	3	.100
	20.0	6.33 (5.11-7.00)	3.47 (2.89-4.00)	2.86 (2.22-3.50)	3	.100
	40.0	5.93 (5.00-6.50)	3.32 (2.78-3.80)	2.61 (2.22-2.90)	2	.057
Sibling Control, N = 4	5.0	12.33 (9.10-17.20)	4.58 (3.70-5.20)	7.73 (4.70-12.00)	2	.057
	20.0	10.8 (9.00-11.10)	4.25 (3.60-4.80)	6.55 (4.70-5.80)	0	.014
	40.0	9.48 (9.00-10.10)	4.05 (3.60-4.30)	5.43 (4.70-5.80)	0	.014
U^2		0	2	0		
p^2		.014	.057	.014		

1. Columns U and p are the values of the Mann Whitney U Test (two-tailed) and probability that light and dark phases are the same.
2. Rows U and p are the values of the Mann Whitney U Test (two-tailed) and probability with a 20-minute criterion that genetically obese and sibling controls are the same.

Table 14. Mean Satiety Ratio (Cal/min)

	Criterion (min.)	Both Phases	Light Phase	Dark Phase	U^1	p^1
Genetically Obese, N = 4	5.0	23.8 (19.6-35.2)	26.2 (19.9-32.1)	23.2 (16.3-40.1)	5	.243
	20.0	22.8 (18.1-33.5)	25.0 (19.9-30.6)	24.3 (15.6-39.4)	5	.243
	40.0	21.6 (17.7-33.4)	23.8 (19.3-30.6)	22.0 (16.3-39.4)	4	.171
Sibling Control, N = 4	5.0	32.3 (25.6-38.0)	41.3 (24.8-56.9)	25.1 (20.5-32.8)	2	.057
	20.0	30.6 (23.7-35.3)	40.2 (25.0-55.5)	22.8 (18.1-27.4)	2	.057
	40.0	28.9 (25.7-34.0)	39.1 (25.0-55.4)	20.1 (16.4-25.6)	1	.027
U^2		3	2	7		
p^2		.100	.057	.445		

1. Columns U and p are the values of the Mann Whitney U Test (two-tailed) and probability that light and dark phases are the same.
2. Rows U and p are the values of the Mann Whitney U Test (two-tailed) and probability with a 20-minute criterion that genetically obese and sibling controls are the same.

Both genetically obese and normal rats consumed approximately the same amounts of food during the periods when the lights were off ($p=.557$, Mann Whitney U Test, two-tailed, Mendenhall, 1971), but when the lights were on, mean intake in the genetically obese rats was 70.1 per cent greater than in the controls, and this difference was statistically significant ($p=.014$, Mann Whitney U Test, two-tables Mendenhall, 1971). Thus the above normal intake in the "fatty" rats in this experiment resulted from increased intake during the light phase.

Mean daily meal size (dark and light phases averaged together) was 120.8 per cent greater in the genetically obese rats than in the controls. In both genetically obese and control animals there was no statistically significant difference between dark and light phase meal sizes (see Table 12).

During the dark phase the "fatty" rats consumed approximately the same amount of food as controls, but meal sizes were approximately double that of the "slims." The "fatties" therefore consumed approximately one-half the number of meals during the dark phase (see Table 13), and as a result the mean ratio of the interval following the meal to the meal size (satiety ratio) was approximately the same in both "fatties" and "slims" during the dark phase (see Table 14).

During the light phase the meal pattern in the genetically obese rats was approximately the same as during the dark phase, and the result was a total intake approximately equal to that during the dark phase. Meal sizes, however, were slightly reduced (7.0 per cent) and meal number was increased (18.2 per cent). These differences were not statistically significant. The normal controls (slims) during the light phase consumed

approximately one-third fewer meals of equal size to those during the dark phase, and hence intake was approximately one-third less then during the dark. Therefore the "fatties" maintained the same satiety ratio during the light phase as during the dark phase ($p=.243$, Mann Whintey U Test with 20-minute criterion, two-tailed, Mendenhall, 1971). The slims increased their mean satiety ratio during the light phase although this difference was statistically significant only with the 40-minute criterion (See Table 14).

Thus the hyperphagia in the genetically obese rats appears to at least partially result from the excess food intake during the normally quiescent part of the day when the lights are on. This is caused by a failure to normally increase intermeal interval following meals of a given size during this period. While all meal sizes in the genetically obese are enclarged, the enlarged meals are compensated by proportionately longer intermeal intervals during the dark phase, and hence the "fatty" rats do no eat sooner than normals after meals of a given size during this period. Therefore, the satiety ratios in "fatties" and "slims" during the dark phase are the same ($p=.445$, Mann Whitney U Test, two-tailed, Mendenhall, 1971). During the light phase, however, the "fatty" animals eat sooner after meals of a given size than the "slims" and hence their satiety ratios are significantly lower (see Table 14).

Discussion

The meal pattern of the "fatty" rat differs in three ways from the meal pattern of its normal weight sibling. First, the "fatty" rat eats

much bigger meals. Second, during the period when the lights are on, the "fatty" does not lengthen the interval following these large meals in the same proportion as would be expected in normal rats and hence eats sooner than the normal rat after meals of a given size. This results in the third difference in the meal pattern of the "fatty" rat, the increased intake during the normally quiescent portion of the daily activity cycle.

The meal pattern of the "fatty" rat in certain ways resembles the meal pattern of the VMH-lesioned rat, but in other ways is quite different. Both genetically obese and VMH-lesioned rats eat larger meals. The VMH-lesioned rat shows the greatest increase in meal size immediately following the lesion during the period when body weight gain is greatest (Becker & Kissileff, 1974). When the VMH-lesioned rat becomes obese and its rate of weight gain is reduced, meal size is only slightly greater than before the lesion (Becker & Kissileff, 1974; Teitelbaum & Campbell, 1958; Thomas & Mayer, 1968). Since the genetically obese rats studied in the present investigation weighed considerably more than the sibling controls, but their mean rate of weight gain was approximately the same, these "fatty" rats more closely resembled obese VMH-lesioned than VMH-lesioned rats immediately following the lesion. Obese VMH-lesioned rats do not usually consume large meals. Therefore the meal pattern of the obese but not dynamic VMH-lesioned rat and the genetically obese animal are different in regard to meal size.

The differences in meal size in the obese "fatty" and the VMH-lesioned rat might be explained in the following way. It has been suggested that body lipid content may act to control food intake through some type of feedback mechanism, and thus when fat cells begin to fill and distend with lipid as a result of excess caloric intake, inhibitory signals

coming from the fat bring about a reduction in food intake (Liebelt, Bordelon,& Liebelt, 1973). This proposed feedback mechanism may act on or through the ventromedial hypothalamus (Hoebel & Teitelbaum, 1966) to reduce intake by reducing meal size (Kissileff & Quartermain, 1973). Thus, in animals with either a dysfunction or destruction in this area, a higher level of body lipids might be required to inhibit food intake and reduce meal size. Furthermore, the feedback signal and its ability to inhibit food intake may depend not only upon the total body lipid content, but also upon its morphology. Obesity in lesioned and fatty rats is morphologically different. In the "fatty" obesity is accompanied by both hyperphasia and hypertrophy in the adipose depots while obesity in the VMH-lesioned animal is accompanied only by hypertrophy(Johnson, Zucker, Cruce,& Hirsh, 1971). As the genetically obese rat gains weight both existing adipocytes fill up and new adipocytes are created since these animals are deficient in stopping cell proliferation in the adipose tissue (Johnson et al., 1971). As the lesioned rat gains weight only existing adipocytes fill up and become enlarged with lipid. Although the exact relationship between body fat stores and feeding behavior is not completely understood, it is suggested that not only the total amount of body fat but also its morphology may be important in influencing feeding behavior.

Both obese VMH-lesioned rats and genetically obese rats distribute their food intake approximately equally between the dark and light periods. This alteration in meal pattern has been clearly shown in several previous studies (Balagura & Devenport, 1970; Becker & Kissileff, 1974; Brooks et al., 1946; Kakolewski et al., 1971) of feeding behavior of VMH-lesioned rats because the control animals in contrast to the lesioned rats consumed

approximately 40 per cent of their total intake during the period when the lights were on, and the difference in the percentage of light phase intake between control and genetically obese rats was not as great as in the studies of VMH-lesioned rats. However, in some of these previous studies (Becker & Kissileff, 1974; Brooks et al., 1946; Kakolewski et al., 1971) lesioned and control rats were of a different strain and sex from the rats in the present investigation. These differences could be an explanation for the higher percentage of light phase eating observed in control animals in this study. Meal patterns of both male and female VMH-lesioned and control animals have been studied and compared (Balagura & Devenport, 1970). It was found that the VMH lesion increased light phase intake in rats of both sexes. Male control animals, however, consumed a higher percentage of their total daily intake during the light period than female control animals. In the present study, however, the increased light phase eating observed in control animals appears to be the result of the difference in strain since it has been found that female rats of the same strain exhibit approximately the same distribution of light and dark phase feeding as the animals studied here (Grinker, unpublished observation 1973).

The observation that both VMH-lesioned and genetically obese animals distribute their food intakes equally between the dark and light phases suggests the possibility of a generalized loss of nychthemeral periodicity in hyperphagia. There are several possible reasons that overeating could be the result of an increase in light phase feeding. First, food intake during the dark phase may normally be at or near a maximum, and for hyperphagia to occur additional food must be consumed at times when little is normally eaten. However, under certain conditions animals are

capable of consuming more food during dark than is normally eaten by VMH-lesioned rats during this period (Kakolewski, unpublished observation--see Kakolewski et al., 1971), and therefore it is probable that food intake during dark is probably not at or near a "ceiling." Second, it is possible that it is most convenient, efficient or least aversive if hyperphagic animals distribute their food intake evenly throughout the 24-hour period. The consumption of large quantities of food over a relatively short period of time might cause gastrointestinal distress analogous to that which occurs in specific aversion behavior (Rozin & Kalat, 1971). A ceiling effect is unlikely in view of the results of the next experiment.

Third, it is possible that both VMH-lesioned and genetically obese rats suffer from a primary loss of nychthemeral periodicity in the normal cycle of lipolysis and lipogenesis and this is the cause of the overeating. While the neuroendocrine mechanism of this cycle is presently unknown, it has been speculated by LeMagnen and co-workers (1973) that it might possibly be at least partially the result of the destruction of the efferent sympathetic activity involved in inhibitory control of the insulinosecretory response to food. There is accumulating evidence that VMH lesions cause a primary disturbance in the neural control of the pancreatic beta cell responsiveness to food (Frohman & Bernardis, 1968; 1969; Hales & Kennedy, 1964; Han & Frohman, 1970; Hustvedt & Løvø, 1972; Steffens, 1969; 1970). In the VMH-lesioned rat during the dark phase a high rate of insulinosecretion to food causes an exaggeration of the lipogenesis normally present in intact rats and results in greater than normal intake of food. This process appears to continue into the light phase and the VMH-lesioned rat continues to overeat during the light phase with the same pattern as during the dark. The genetically obese rat is hyperinsulinemic (see Bray &

York, 1971a, for review), and it is possible that a disturbance similar to that which is believed to occur in VMH-lesioned (LeMagnen et al, 1971) rats is also the cause of the hyperphagia in the genetically obese animal. However, obesity is usually accompanied by hyperinsulinemia, and there is no evidence to suggest that the genetically obese animals have a primary defect in the responsiveness of their beta cells.

This experiment points to the possibility of a generalized loss of nychthemeral periodicity in hyperphagia since both genetically obese rats and VMH-lesioned animals exhibit this alteration in their feeding pattern. The results of this experiment suggest that hyperphagia could be severely reduced or eliminated if VMH-lesioned or genetically obese rats were restricted to feeding for only one-half of the day, and that lactating rats would exhibit loss of nychthemeral periodicity before making other alterations in intake patterns, if this deficit is a common mechanism for increased food intake.

Experiment 3. Meal-Taking Behavior in Lactating Rats.

Food intake in the lactating rat equals and exceeds the hyperphagia of the VMH-lesioned animal (Kennedy, 1953). When VMH lesions are made in lactating rats, food intake is unaffected and obesity does not occur until after the pups are weaned (Kennedy, 1953). This suggests that "something about the process of lactating resulted in the production of a transitory, functional (i.e. reversible) hypothalamic 'lesion' of the type that causes obesity in the non-lactating animal" (Tepperman, 1973, p. 95), and this is the cause of increased food intake.

Alternatively, it is possible that the increased food intake is the result of causes other than a disturbance in the normal hypothalamic function (e.g. changes in the animal's hormonal state), and that the only reason that VMH lesions do not produce further increases in food intake is because food intake may have already reached the animal's physiological maximum, and thus cannot be further increased as a result of the lesions.

Nothing is known about the alterations in meal patterns which accompany hyperphagia in the lactating rat. It is known that food intake gradually increases during lactation (Kennedy, 1953; Ota & Yokayama, 1967). If the alterations in the meal pattern of the lactating rat differ from those which accompany VMH lesions, it would demonstrate that the two forms of hyperphagia do not share a common mechanism.

This experiment was conducted to determine what alterations in meal patterns accompany hyperphagia in lactating rats. Since it has been shown that there are quantitative differences in the way hyperphagic VMH-lesioned rats consume different diets (Teitelbaum & Campbell, 1958; cf. Becker & Kissileff, 1974 and experiment 1), the meal patterns of lactating rats were examined using both a highly palatable milk (Becker & Kissileff, 1974) and solid laboratory chow pellets (Noyes).

Method

Continous records of meal patterns were obtained before parturition, during lactation, and for 5 - 20 consecutive days following the weaning and removal of the litter. Both solid and liquid diets (see diets- General Methods) were used.

Animals

Seven female Wistar rats weighing a mean of 360 (R=278-425) g at parturition and a mean of 252 (R=199-369) g on the day following parturition were used. The rats were obtained late in pregnancy from the supplier (Carworth Labs, Nyack, New York).

Housing

During the period of lactation the size of the living chambers was enlarged to accomodate the dam and the litter. Animals on the milk diet were at this time housed in a chamber measuring, inside, 19.1 x 47.0 x 20.3 cm high. In one wall two holes were drilled and spouts containing the diet and water were positioned just outside of these holes (see apparatus: liquid diets - General Methods). One half of the chamber (opposite to wall with holes) was completely covered

with animal bedding (Iso-Dri). Care was taken to make sure that the bars in the cage floor near the spout remained clear of the bedding so that the rats would make contact with them while drinking. Each day the lick-to-intake ratio was calculated to determine that proper contact was made and that the drinkometer was operating properly. The days on which the lick-to-intake ratio did not fall between .0015 and .0050 ml per lick the meal pattern data were not used. As a result of this 5 days during lactation were eliminated in Preg 9, 2 in Preg 10, 1 in Preg 11, 2 in Preg 12, 2 in Preg 13, 5 in Preg 14, and 5 in Preg 15.

Animals on the solid diet were housed in the high walled box (see apparatus: solid diet - General Methods) equipped with an eatometer (see apparatus: solid diet - General Methods). Attached to this box was a masonite chamber measuring inside 29.2 x 21.6 x 16.5 cm high with a floor completely covered with animal bedding. Experience with chambers of smaller dimensions resulted in the death of some of the pups, a phenomenon previously noted by Wang (1924).

Sequence of Procedures

For seven to one days before parturition body weight and food and water intakeswere measured daily and in addition, meal patterns were recorded (see automatic recording and feeding patterns - General Methods).

Litters were standardized at ten pups on the first day following parturition. In those litters in which there were more than ten pups, the number of pups was reduced to ten. In those

litters in which there were fewer than ten pups, attempts were made to add extra pups when available. During lactation due to the death of some pups, the litter weights, number of pups, and the weight gain of the litters were not uniform (see Table 15). When possible attempts were made to add extra pups from other litters to maintain ten pups in each litter by replacing pups which died with pups of the same age. Food and water intakes were made daily for 18 consecutive days. From the 18th to 21st days of lactation, no meal pattern data were collected since the pups were both suckling as well as consuming the experimental diets. The litter was weaned and the pups removed on the 21st day following parturition. Meal pattern data as well as body weight and water intake measurements were collected from the dam for an additional 20 days in Preg 9, Preg 10, Preg 11, Preg 13, and Preg 15; 10 days in Preg 12; and 5 days in Preg 14.

Results

Food intake and meal size increased gradually during the first 15 days of lactation. Meal number decreased slightly. Despite the high intake, predominately nocturnal feeding persisted. Following weaning, intake and meal parameters returned to pre-parturition levels. Results were similar with both liquid and solid diets. Meal pattern profiles for a typical animal on the liquid diet (Preg 11) and a typical animal on the solid diet (Preg 15) are shown in Figures 10 and 11.

The results will be presented describing meal patterns at each stage of the experiment and will be preceded by a general description of the distribution of intermeal intervals and the

Figure 10. Meal pattern profile of a representative rat fed milk before,during, and following lactation. For detailed description, see Figure 1. In addition to the information about Figure 1 applicable here, body weight of the litter is also shown.

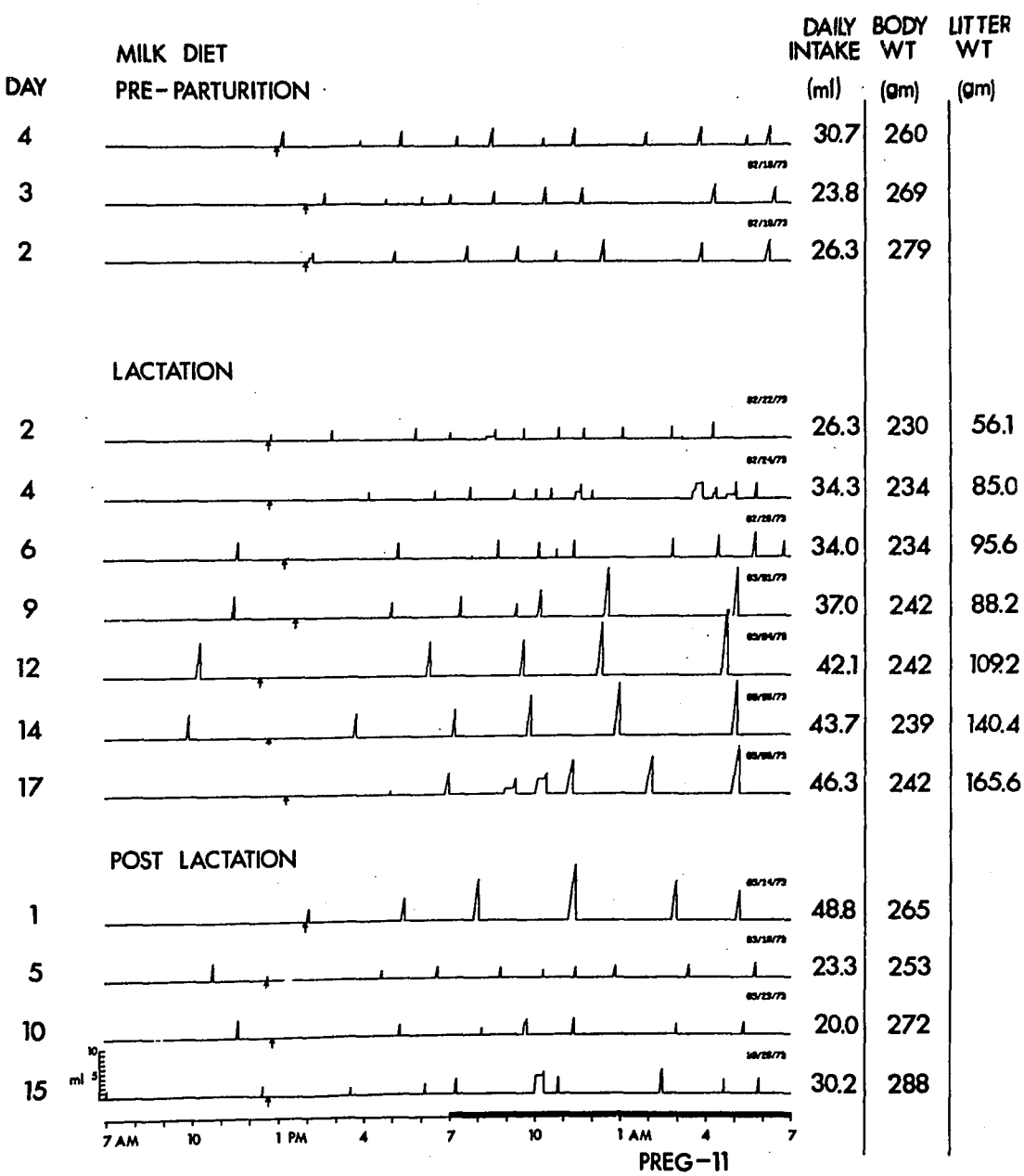
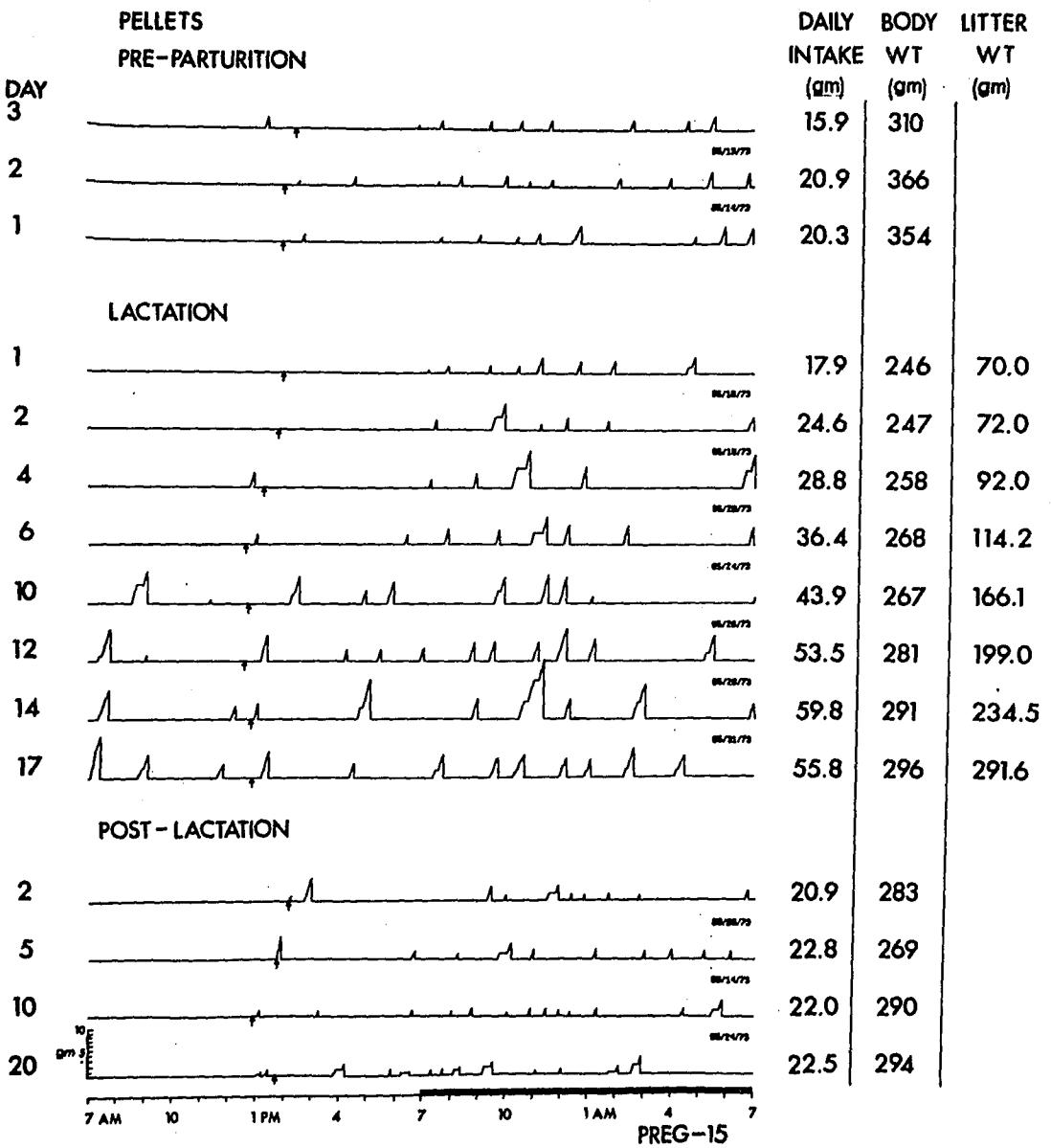

MILK DIET
PRE-PARTURITION
DAY
DAILY INTAKE (ml)
BODY WT (gm)
LITTER WT (gm)
4 30.7 260
3 23.8 269
2 26.3 279
LACTATION
2 26.3 230 56.1
4 34.3 234 85.0
6 34.0 234 95.6
9 37.0 242 88.2
12 42.1 242 109.2
14 43.7 239 140.4
17 46.3 242 165.6
POST LACTATION
1 48.8 265
5 23.3 253
10 20.0 272
15 30.2 288
ml
7 AM 10 1 PM 4 7 10 1 AM 4 7
PREG-11

Figure 11. Meal pattern profile of a representative rat fed pellets before, during, and following lactation. For a detailed description, see Figure 1. Body weight of the litter is shown at right.


PELLETS
PRE-PARTURITION
DAY
DAILY INTAKE (gm)
BODY WT (gm)
LITTER WT (gm)
3
15.9
310
2
20.9
366
1
20.3
354
LACTATION
1
17.9
246
70.0
2
24.6
247
72.0
4
28.8
258
92.0
6
36.4
268
114.2
10
43.9
267
166.1
12
53.5
281
199.0
14
59.8
291
234.5
17
55.8
296
291.6
POST - LACTATION
2
20.9
283
5
22.8
269
10
22.0
290
20
22.5
294
10 gm
7 AM
10
1 PM
4
7
10
1 AM
4
7
PREG-15

rationale for choosing the 20-minute criterion to describe the results.

Distribution of Intermeal Intervals

Intermeal intervals as in previous studies (Kissileff, 1970; experiment 1) were distributed bimodally during most of the conditions with a large peak whose maximum ranged from 60 to 140 minutes depending on the animal and condition and a small peak at 5 minutes separated by a trough of 10 to 40 minutes (see Figures 12 and 13).

There were a number of important quantitative differences in the general pattern of the distribution of intermeal intervals across diets and during lactation which can be seen in Figures 12 and 13. These will be described with respect to the small peak at 1-10 minutes, the large peak (40-180 minutes), and the valley (10-40 minutes) in the distributions of intermeal intervals. First, before parturition and early in lactation rats fed milk interrupted feeding for periods of 10 minutes or less with frequencies equivalent to those of rats fed pellets (cf. Figures 12 and 13, open and closed squares). Interruptions of less than 10 minutes constituted a mean of 0.68 (R=0.0-2.0) per day or a mean of 5.74 (R-0.0-14.0) percent of the total number of interruptions for the rats eating milk, during the first 5 days of lactation. Comparable figures for the rats eating pellets were a mean of 1.36 (R=0.33-2.0) interruptions per day or a mean of 12.4 (R=4.5-19.0) percent of the total number of intervals. However, during lactation the number of short interruptions

Figure 12. Mean daily distribution of intermeal intervals per day for the 4 animals fed milk. Each point is the mean daily number of intervals exceeding the absciassa value of the preceding point and less than or equal to its own abscissa vaue. For example, the 60-min point is the number of intervals from 40.1 min to 60.0 min. Eight points, each representing 5-min intervals in length are plotted up to 40 min. One point is plotted for each 20-min interval from 40 to 400 minutes. The last point includes the number of intervals exceeding 400 min.

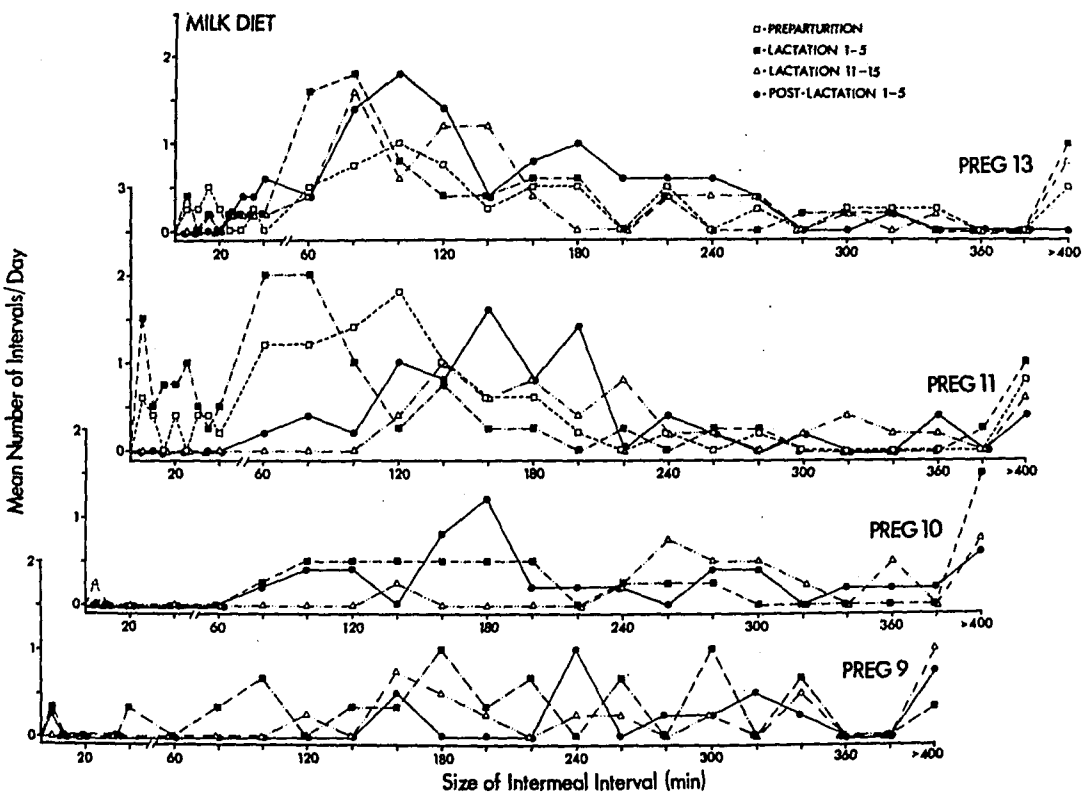
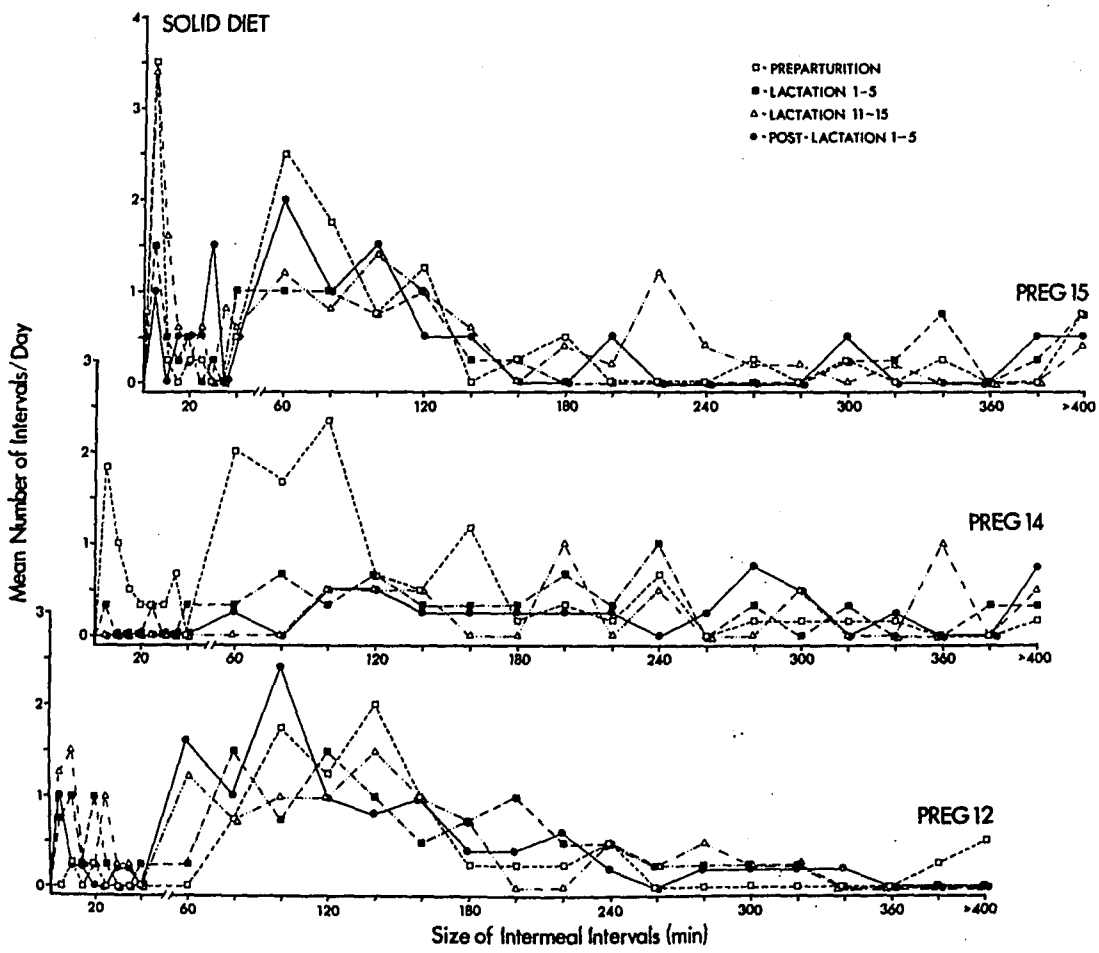

MILK DIET
□-PREPARTURITION
■-LACTATION 1-5
△-LACTATION 11-15
●-POST-LACTATION 1-5
PREG 13
PREG 11
PREG 10
PREG 9
Mean Number of Intervals/Day
Size of Intermeal Interval (min)
20
60
120
180
240
300
360
>400

Figure 13. Mean daily distribution of intermeal intervals for animals fed pellets. For detailed description, see Figure 12.


SOLID DIET
PREPARTURITION
LACTATION 1-5
LACTATION 11-15
POST-LACTATION 1-5
PREG 15
PREG 14
PREG 12
Mean Number of Intervals/Day
Size of Intermeal Intervals (min)
20
60
120
180
240
300
360
>400

(less than 10 minutes) vanished completely in the rats eating milk (see Figure 12, open triangles) while the number of short interruptions increased to 2.75 and 5.0 interruptions per day or 20 and 30 percent respectively of all interruptions in rats Preg 12 and 15 during the 11th-15th days of lactation. Preg 14 (also fed pellets) did not show this increase but she also had the smallest litter due to high infant mortality.

The number of least-frequently occurring intervals in the valley (10-40 minute range) was also different from early to late in lactation with the milk diet. In three of the four rats (Preg 9, 10, 11) these intervals disappeared completely during the 11th-15th days of lactation but in the fourth rat (Preg 13) they remained unchanged from early in lactation. In the rats on pellets the number of least frequently occurring intervals was inconsequentially higher during the 11th-15th days of lactation than during the first five days of lactation in Preg 12 and 15, and disappeared completely in Preg 14 (see Figure 13, open triangles). The number of intervals between 10 and 40 minutes rose from a mean of 1.75 to 2.0 and from 2.0 to 2.8 in Preg 12 and 15 respectively from the first five days of lactation through days 11-15.

Finally we consider the most frequently occurring interval range (40-180 minutes). In the animals fed pellets these remained stable during the course of lactation and following lactation and with the exception of Preg 14 were almost identical to their pre-parturition distribution. In Preg 14 the number of these intervals became distributed more randomly during lactation and the

number of these intervals dropped from 7.66 per day before parturition to 1.5 per day during the 11th-15th days of lactation. Although the number of intervals greater than 180 minutes was increased from 3.32 before parturition to 3.50 during the 11th-15th days of lactation, the percentage of intervals in this range increased from 31.9 percent before parturition to 70 percent during lactation. In other words intermeal intervals became very long during lactation in this rat. In rats fed milk the intermeal intervals became distributed more randomly during lactation and only in one rat, Preg 13, was there any trace of the normal peak which occurred at 80 minutes (see Figure 12, open triangles).

In the five days immediately after lactation (see Figures 12 and 13, closed circles) and during subsequent periods after lactation the distribution of intermeal intervals was affected in the following manner. First, short interruptions (less than 10 minutes) which had increased during lactation in two of the animals fed pellets (Preg 12, 15) decreased to a mean of 1.2 and 1.0 intervals per day respectively (10.0 and 8.3 percent of all intervals) but were unchanged from their values during lactation in the other rats. As a result the number of intervals shorter than 10 minutes in length was less than before parturition in Preg 15, but remained between the values of the pre-parturition and lactation periods in Preg 12. Second, in all rats the number of least frequently occurring intervals (10-40 minute range) did not change from the later periods of lactation. Third, in all of the animals fed pellets the most frequently occurring intervals (40-180

minute range) did not change. In Preg 12 and 15 it remained identical to the distribution before parturition and during lactation. In Preg 14 the distribution of these intervals which had become random during lactation remained random. In two of the rats fed milk (Preg 11, 13) the distribution of intervals between 40 and 180 became less randomly distributed and a peak appeared at 100 (Preg 13) and 160 minutes (Preg 11). In the other two animals fed milk the distribution of inervals within this range did not change its shape from lactation and the intervals remain randomly distributed.

Choice of Criterion to define intermeal intervals. In the absence of any physiological data on satiety, Kissileff (1970) has suggested that the nadir in the distribution of intermeal intervals could be used as an indication of a period of time following a meal when satiety is high. The rationale behind this is that meals should be separated from each other by period of satiety, and a time when the animal is least likely to eat, may be a period when satiety signals are at their maximum (assuming such a continuum in satiety signal level exists). From figures 12 and 13, it can be seen that there was considerable variation among individual animals and conditions in the length of the shortest of the least frequently occurrring interruptions between feeding bouts. However, the total number of interruptions between 10 and 40 minutes (from 1 to 2 per day)

comprised only a small percentage of the total number of interruptions between bouts of feeding, and therefore the choice of any criterion between 10 and 40 minutes in length would matter little. For example, in all of the rats fed milk and in one of the rats fed pellets the choice of criterion during the 10th-15th days of lactation makes no difference in either the quantitative results or its interpretation because most of the time there are no interruptions of less than 40 minutes between bouts of feeding. In the other two rats fed pellets the number of interruptions between feedings of less than 40 minutes increased slightly during lactation so that the use of a 40-minute as opposed to a 10-minute criterion during the 10th-15th days of lactation would have the effects of dropping 2.0 and 2.8 meals per day or decreasing the number of meals by 14.6 and 17.5 percent respectively. This quantitative effect did not change the interpretation of the basic phenomena to be reported.

Because of the great deal of individual variation in the least frequently occurring shortest interruptions between feeding bouts and because relatively few interruptions fell between 10 and 40 minutes in all animals and with all conditions, all data in this experiment have been analyzed with an arbitrarily selected 20-minute criterion. The reason for choosing this criterion was that it would enable the easiest comparison with the data of VMH-lesioned rats and genetically obese animals reported in the preceding experiments and in an earlier report (Becker & Kissileff, 1974). Inspection of the data using 10- and 40-minute

criteria produced only quantitative and not qualitative changes in the results.

Meal Pattern Before Parturition

The meal pattern was similar with both diets. The meal pattern of the animals on the liquid diet consisted of a mean of 8.7 (R=7.0-20.4) discrete meals per day. The mean daily meal size was 2.70 (R=2.44-3.15) ml. Meals consumed during the dark phase were a mean of 18.8 (R=14.6-24.0) percent larger than meals consumed during the light phase (see Figure 15). Feeding was predominantly nocturnal and a mean of 70.1 (R=69.6-70.7) percent of the total daily intake was consumed during the dark phase (see Figure 15).

Animals on the solid diet consumed a mean of 10.0(R=9.0-11.8) discrete meals each day. Mean daily meal size was 2.20 (R=1.97-2.54) g. Dark phase meals were 30.7 (R=17.4-44.1) percent larger than light phase meals (see Figure 16). Feeding was predominantly nocturnal and a mean of 77.2 (R=64.8-90.5) percent of the total daily intake was eaten while the lights were off.

Meal Pattern During Lactation

During lactation food intake increased gradually with both diets until it was approximately double the level before parturition. The increased intake during lactation was almost exclusively the result of dramatic progressive increases in meal size which occurred with both diets (see Figures 15 and 16). Animals fed the milk diet did not disturb the nychthemeral periodicity of feeding at any time during lactation. Animals on the solid diet, however, increased the percentage of total intake consumed during the light phase.

Figure 14. Means and ranges of food intakes for animals fed milk diet (top) and pellets (bottom) before, during, and following lactation. The darkened portion of each bar indicates mean intake during the dark phase. The light portion indicates mean intake during the light phase. The vertical lines extending above and below each bar on the figure represent the range of each animal's mean intake for that period. The height of the slashed portion of the compound bar shows the total number of meals.
*Data collected only in Preg 12.
/Data collected only in Preg 15.

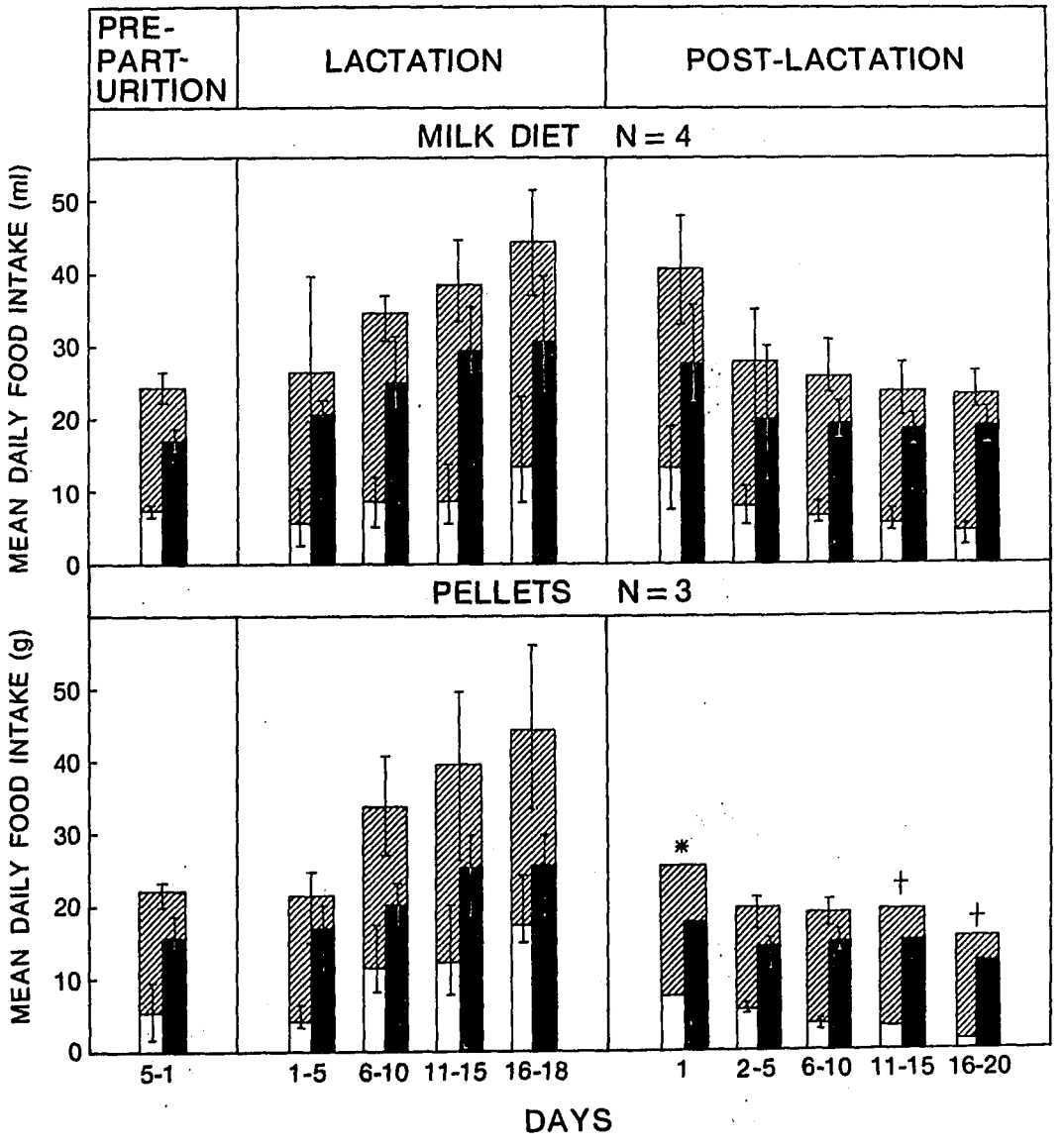

PRE-PART-URITION
LACTATION
POST-LACTATION
MILK DIET N = 4
PELLETS N = 3
MEAN DAILY FOOD INTAKE (ml)
MEAN DAILY FOOD INTAKE (g)
0
10
20
30
40
50
*
†
†
5-1
1-5
6-10
11-15
16-18
1
2-5
6-10
11-15
16-20
DAYS

Figure 15. Means and ranges of meal sizes and mean daily number of meals in the animals fed milk before, during and following lactation. The bars in the top half of the figure represent the mean meal size for the entire day for each of the periods. The vertical line in the center of each bar, extending above and below the top of the bar indicates the range of mean daily meal sizes for that period. The vertical line on the left of each bar represents the range of light phase meal sizes for that period. The horizontal line in the center of this line indicates the mean light phase meal size. The vertical line on the right hand side of each bar represents the range of dark phase meal sizes for that period. The horizontal line in the center of this line indicates the mean light phase meal size. The darkened portions of the bars in the bottom half of the figure indicate the mean number of dark phase meals for each period. The light portions indicate the mean number of light phase meals. The vertical lines extending above and below each bar indicate the range of each animal's mean daily number of meals for that period.

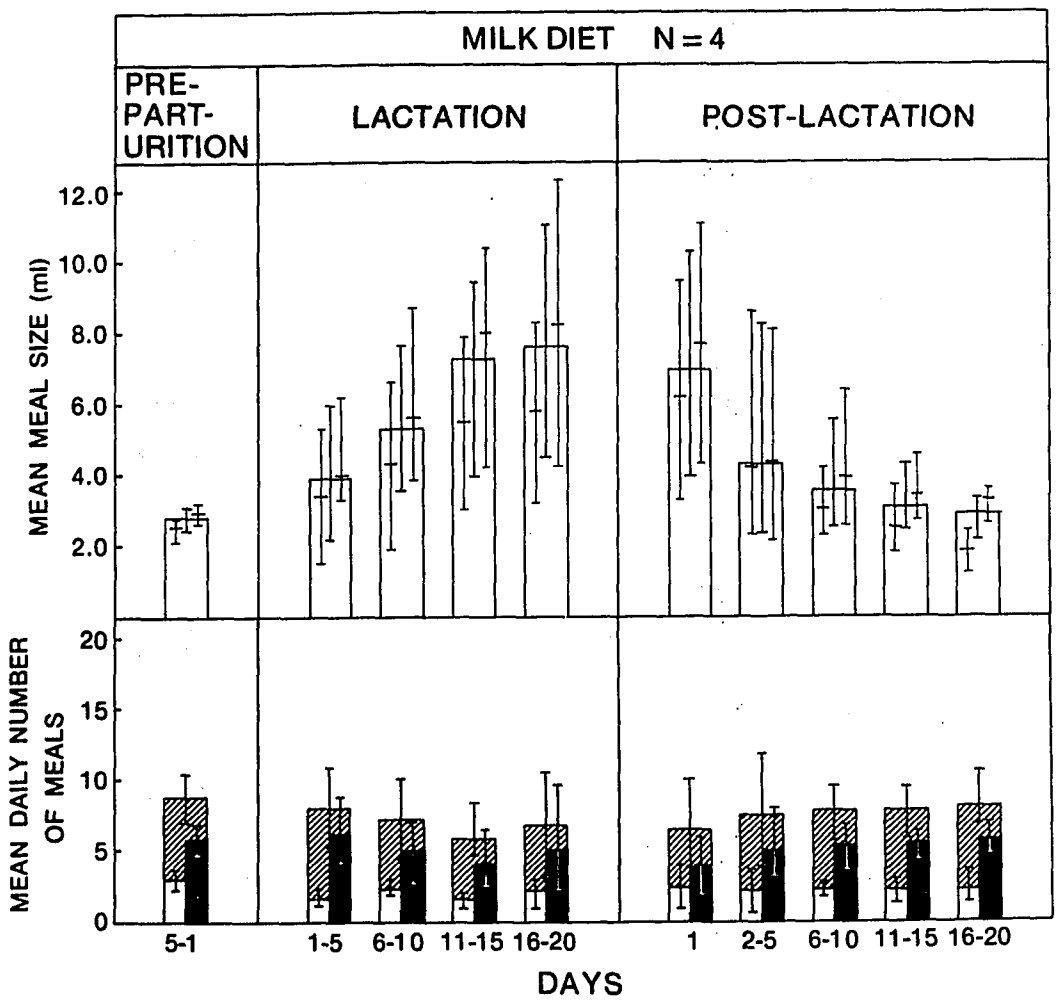
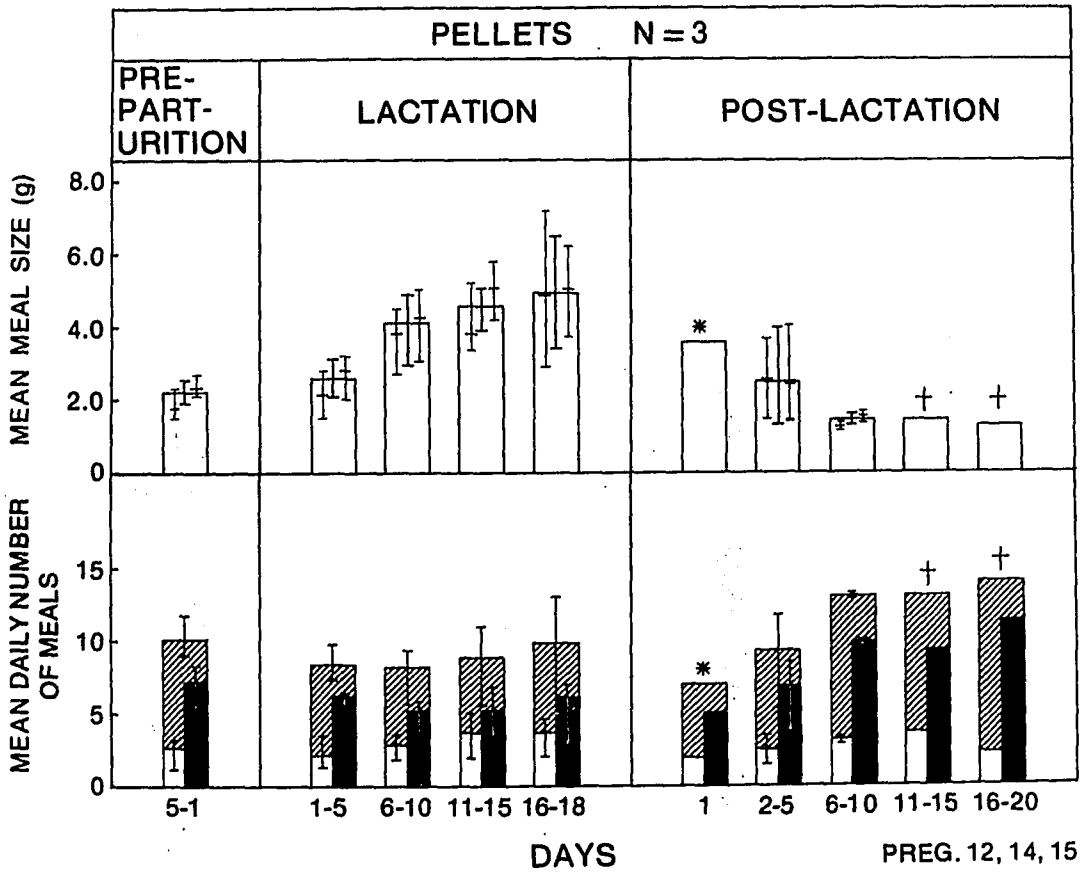

MILK DIET N = 4
PRE-PART-URITION
LACTATION
POST-LACTATION
MEAN MEAL SIZE (ml)
12.0
10.0
8.0
6.0
4.0
2.0
MEAN DAILY NUMBER OF MEALS
20
15
10
5
0
5-1
1-5
6-10
11-15
16-20
1
2-5
6-10
11-15
16-20
DAYS

Figure 16. Means and ranges of meal size and mean daily number of meals in the animals fed pellets before, during, and following lactation. For detailed description, see Figure 15.
*Data collected only in Preg 12.
/Data collected only in Preg 15.


PELLETS N = 3
PRE-PART-URITION
LACTATION
POST-LACTATION
MEAN MEAL SIZE (g)
8.0
6.0
4.0
2.0
0
MEAN DAILY NUMBER OF MEALS
15
10
5
0
5-1
1-5
6-10
11-15
16-18
1
2-5
6-10
11-15
16-20
DAYS
PREG. 12, 14, 15

Milk Diet. Food intake increased gradually during lactation (see Figure 14) and reached its maximum between the 16th-18th days of lactation. At that time intake was a mean of 68.4 (R=12.4-99.5) percent greater than between the 1st-5th days of lactation. In Preg 11 and 13, the two rats fed milk in which pre-parturition data was collected food intake during the 16th-18th days of lactation was a mean of 86.9 (R=78.7-95.2) percent greater than it was before parturition. Intakes during the light and dark phases increased by equal percentages during lactation and the percentage of the total intake consumed during the dark phase did not change during lactation. A mean of 73.7 (R=68.7-88.3) percent of the total daily intake was consumed during the dark phase between days 1 and 5 of lactation and a mean of 70.7 (R=55.3-81.4) percent between days 16 and 18. Mean daily meal size gradually increased during lactation until between the 16th-18th days of lactation it reached a mean of 95.9 (R=65.9-181.9) percent larger than between 1st-5th days of lactation. Meal sizes during the dark phase as before parturition exceeded meal sizes during the light phase (see Figure 15) and between the 16th and 18th days of lactation mean meal sizes during the dark phase exceeded mean meal sizes during the light phase by a mean of 42.6 (R=11.3-104.1) percent. The daily number of meals declined by a mean of 28.8 (R=6.7-44.2) percent between the first five days of lactation and the 10th-15th days of lactation and then increased slightly. Between the 16th-18th days of lacation the mean daily number of meals was a mean of 14.1 (R=50.0 less, 16.7 greater)

percent less than during the first five days of lactation (see Figure 15). However, it is possible that the increase in meal number between days 16-18 was the result of occasional nibbling of the diet by the pups.

Pellets. Food intake increased gradually and maximum intake was reached between the 16th-18th days of lactation. However, the meal pattern data from this period was occasionally contaminated by the pups eating the diet. Intake between the 16th-18th days of lactation exceeded intake during the first five days of lactation by a mean of 107.4 (R=79.0-131.7) percent and intake before parturition by a mean of 95.6 (R=42.5-181.5) percent. The percentage of the total daily intake consumed during the dark phase was unchanged from before parturition during the first five days of lactation (mean of 79.2 (R=74.7-84.5) percent), but during subsequent periods decreased. Between the 10th and 15th days of lacation a mean of only 65.3 (R=58.4-70.8) of the total daily intake was consumed during the dark phase and between the 16th and 18th days of lactation a mean of 60.1 (R=54.9-69.6) percent.

As with the milk diet meal sizes gradually increased during lactation and between the 16th and 18th days of lactation were a mean of 89.6 (R=33.9-206.1) percent larger than during the first five days of lactation and a mean of 124.1 (R=33.7-229.4) percent larger than before parturition. Mean meal sizes during the dark phase were always larger than during the light phase (see Figure 16). The effect of lactation upon meal frequency varied considerably among individual animals. In one rat (Preg 14) it declined throughout

lactation and was 57.7 percent less than before parturition between days 16-18 of lacation. However, this rat also lost most of its litter (see Table 15). In the other animals it remained virtually unchanged during the first 10 days of lactation and then increased. In these (Preg 12 and 15) meal frequency was 44.4 and 21.2 percent greater respectively than it was before parturition between days 16-18.

Meal Pattern Following Lactation

On the first day following the removal of the litter, food intake and meal size declined only slightly from the levels during the last period of lactation in all rats (see Figures 14, 15, and 16). Within ten days, however, all rats were eating approximately the same amount as before parturition (see Figure 14). Meal size declined to the level before parturition in all of the animals on both diets also within ten days (see Figures 15 and 16) except Preg 11. In Preg 11 meal sizes did not return to the level before parturition and remained elevated by a mean of 36.7 percent (days 15-20 post lactation).

Litter Size, Weight, and Weight Gain

Only three of the seven rats (Preg 9, 10, 15) maintained 10 pups in their litters until the 21st day of lactation. The losses sustained by the dams are shown in Table 15. There were no clear effects of litter size on the intake and meal parameters.

Table 15. Litter Size and Weight, Intake, and Meal Parameters of the Dams

Dam	Diet	Litter Size (Number of Pups) Day 1	Litter Size (Number of Pups) Day 21	Litter Weight (g) Day 1	Litter Weight (g) Day 21	Intake Lactation Days 10-15 g or ml	Meal Parameters Lactation Days 10-15 Meal Size (g or ml)	Meal Parameters Lactation Days 10-15 # of Meals
Preg 9	Milk	10	10	101.5[1]	267.7	34.8	8.20	4.25
Preg 10	Milk	10	10	57.7	233.0	40.1	9.42	4.25
Preg 11	Milk	7	5	52.5	193.4	44.7	7.45	6.0
Preg 13	Milk	12	9	66.9	173.3[2]	33.4	3.97	8.4
Preg 12	Pellets	10	5	62.2	198.0	42.8	3.89	11.0
Preg 14	Pellets	7	4	43.8	109.4[2]	26.7	4.86	5.5
Preg 15	Pellets	12	10	76.0	353.0	50.3	5.03	10.2

[1] Day 3 of Lactation

[2] Day 18 of Lactation

Discussion

During lactation the rats in this experiment doubled their food intake. The increase was accompanied by two alterations in the normal meal pattern. The first alteration was an enormous increase in the size of individual meals without a compensatory decrease in the daily meal frequency. This alteration occurred with both diets. The second alteration was an increase in the percentage of light phase eating. This alteration, however, was limited only to the animals consuming the solid diet.

Therefore the failure of lactating rats to terminate ongoing feeding appears to be the major cause of the overeating. There are several possible explanations which could account for the enlarged meals. A likely explanation is that the enlarged meals result from changes in the animal's hormonal state. Associated with lactation is a reduction in the levels of progesterone and estrogen, and the release of prolactin and oxytocin. Only the effects of decreased levels of estrogen on meal patterns have been investigated. It has recently been shown that the mild hyperphagia following ovariectomy (Mook, Kenney, Roberts, Nussbaum, & Rodier, 1972; Rodier, 1972; Wade & Zucker, 1969) is accompanied by an increase in meal size (Kenney & Mook, 1974). The increase in food intake and meal size, however, following ovariectomy is considerably less than the increase during lactation which occurred in this study. Thus changes in the levels of other hormones may affect the meal pattern in addition to the decreased levels of estrogen.

It is also possible that the pattern of predominantly large meals was due to the fact that the dam had to remain with its litter a large part of the time, and thus altered its meal pattern because of this. This seems unlikely in view of two observations. First, the percentage of time spent with the litter and the duration of the periods with the litter gradually declines in the post-partum period (Grota & Ader, 1969) while the meal sizes gradually increase. Second, the enlarged meals do not disappear immediately following the removal of the litter but remain for one to two days. This would suggest that the presence of the litter and the need to suckle it does not cause the alterations in the meal pattern.

The data from this experiment must be considered inconclusive in determining whether or not the increased intake in the lactating rat is the result of some form of transitory functional hypothalamic lesion disrupting at least some of the normal functions of the VMH because there are both similarities and differences in the feeding behavior of VMH-lesioned and lactating rats. The feeding behavior of VMH-lesioned rats and lactating rats are similar in the following respects and these similarities would suggest that the increased food intakes might be caused by a common mechanism. First, the percentage increase in intake in both is comparable. The increased food intakes which occurred in the lactating rats in this study were comparable to the hyperphagia of VMH-lesioned rats fed the same diets. The intakes of VMH-lesioned rats fed the milk diet in a previous study (Becker & Kissileff, 1974) were increased a mean of

91.9 percent from pre-lesion during the 4th-10th days post lesion. Lactating rats fed milk in this study ate a mean of 86.9 more between the 16th-18th days of lactation than before parturition.[≠] Comparable increases for the animals fed pellets were a mean of 124.8 percent for VMH-lesioned rats (see experiment 1) and 95.6 percent for the lactating animals. Second, both VMH-lesioned and lactating rats eat meals of abnormally large size. In this study it was shown that meal size in lactating rats are not only several times larger than normal, but even exceeded those of VMH-lesioned rats fed the same diets. Meal sizes of VMH-lesioned rats fed milk increased from pre-lesion a mean of 71.4 percent during the first three days post lesion[ǂ] to a mean of 3.6 (R=3.0-4.4) ml (Becker & Kissileff, 1974). The meal sizes of the lactating rats in this study fed milk during the 16th-18th days of lactation were a mean of 108.2 percent greater than before parturition and averaged 5.6 (R=4.5-6.7) ml[≠]. The comparable increases for the animals fed pellets were a mean of 57.9 percent to a mean of 2.3 (R=1.7-2.9)[ǂ] g for the VMH-lesioned animals* (see experiment 1) and a mean of 95.9 percent to a mean of 4.9 (R=3.4-6.5) g for the lactating rats.

[≠] Preg 11 and 13. Since no pre-parturition data was collected in the other animals fed milk, comparisons between pre-parturition and lactation are made only in these two animals.

[ǂ] 20-minute criterion.

* The maximum increase in meal size occurred between 10th-15th days post lesion at which time meal size was increased a mean of 94.5 percent to 2.8 (R=1.5=4.1) g (with 20-minute criterion) for the comparison with previous study days 1-3 post lesion were used.

The feeding behavior of VMH-lesioned and lactating rats, however, differed in the following ways and these differences indicate that the mechanisms for the increased intake are not identical. While both VMH-lesioned and lactating rats consume large meals, there are differences in the manner in which these meals are consumed with solid diets. VMH-lesions produce approximately a ten-fold increase to 10-16 per day in the number of short (1-10 minutes) interruptions between bouts of feeding (see experiment 1). During lactation the number of these interruptions between feeding bouts does not increase as dramatically (approximately only a two-fold increase to 3-5 per day). In addition following VMH lesions there is approximately a three-fold increase to 1-12 per day in the number of normally least-frequently occurring intervals (10-40 minutes). During lactation there was either a complete disappearance of intervals of this length or only an incidental increase to 2-3 intervals per day. Thus the enlarged meals in the lactating rats are not interrupted as frequently as in the VMH-lesioned rats (cf. Figures 2 and 13, note differences in scale of ordinates).

The normal nychthemeral periodicity of feeding is disrupted in both VMH-lesioned and lactating rats, but both the nature of this disturbance and its probable cause appear to be different. In the VMH-lesioned animal, the disruption is a permanent change in the meal pattern apparently resulting directly from the lesion. Immediately following a VMH lesion, food intake is distributed equally between the light and dark phases (Becker & Kissileff, 1974; experiment 1). This equal distribution of food intake between

the light and dark phases persists even when the animals become obese and their food intakes return to pre-lesion levels (Becker & Kissileff, 1974; Kakolewski et al., 1971). Furthermore, the disruption occurs with both solid (Balagura & Devenport, 1970; Kakolewski et al., 1971; LeMagnen et al., 1973; experiment 1) or liquid diets (Becker & Kissileff, 1974). In contrast, lactating rats exhibit a disruption in nychthemeral periodicity of feeding only when fed a solid diet and only during the periods of lactation when the most intense hyperphagia occurs. This disruption in nychthemeral periodicity of feeding may be caused by the inability of the lactating rat to digest sufficient quantities of the solid diet during the dark phase period. Thus in order to take in sufficient food, the lactating rat eats more during the light phase period. This would suggest that there may be absolute limits to the amount of a particular diet which an animal can consume during a 12-hour period, and when the nutritional requirements of the animal exceed this limit, the only way the animal can increase its food intake is by consuming additional food during the next 12-hour period. Therefore the elimination or severe reduction of hyperphagia by limiting the period of food access may not be an indication of the mechanism causing the hyperphagia.

Furthermore, lactating rats when consuming milk maintain normal mychthemeral periodicity of feeding during the entire period of lactation. This indicates that the disruption in the daily cycle of lipolysis and lipogenesis which has been suggested as the cause of the hyperphagia in VMH-lesioned rats (LeMagnen et al., 1973) probably does not cause the hyperphagia in the lactating rat.

Experiment 4. Independence of the Nychthemeral Periodicity of Feeding and Hyperphagia Following VMH Lesions

Following VMH lesions much of the excess intake of the lesioned rats is consumed during the period when little food is normally eaten (Balagura & Devenport, 1970; Becker & Kissileff, 1974; Brooks et al., 1946; Kakolewski et al., 1971). It has been suggested that VMH-lesioned rats, "are, in fact, hyperphagics because they eat in daytime the same amount of food as in the night" (LeMagnen, 1971, p. 245). The implication of this proposal is that an inhibitory control over light-phase eating has been destroyed by the lesion.

A mechanism has been proposed to account for this phenomenon by LeMagnen and co-workers (1973). They have suggested that a lipostatic mechanism operates within the diurnal cycle of feeding in normal rats as follows. During the dark phase the animals consume an amount of food in excess of their needs. This high consumption of food during the dark phase results in lipogenesis. During the following light phase period lipolysis occurs. This lipolysis then causes a suppression of food intake (LeMagnen & Devos, 1970). Thus the normal rat has a daily cycle of lipogenesis and lipolysis which coincides with the dark and light phases respectively. VMH-lesioned rats persist in lipogenesis during the day, lack this cycle (LeMagnen et al., 1973) and thus "it seems clear this persistence i.e., the lack of lipostatic mechanism, is in lesioned rats a direct cause of the absence of compensatory night-day adjustment of food intake--i.e., the cause of hyperphagia" (LeMagnen et al., 1973, p. 21). Thus according to LeMagnen et al., (1973) the increased feeding during the light phase is the secondary result of a disturbance in the daily cycle of lipogenesis and lipolysis.

Evidence consistent with this hypothesis is the finding that genetically obese rats consume much of their excess food intake during the period when little is normally eaten (experiment 2) although a nychthemeral analysis of their lipolysis-lipogenesis cycle has not been published. Thus, equal intake during the light and dark phases is an alteration in feeding behavior common to at least two different types of hyperphagic rat. These observations suggested that there might be a generalized loss of nychthemeral periodicity in feeding in hyperphagia.

It is also possible, however, at least in the VMH-lesioned rat that the disruption in nychthemeral periodicity of feeding is not the cause of the hyperphagia, but that the disruption and the overeating are independent, or alternatively that the equal dark-light distribution of eating is the cause of the disrupted lipogenesis-lipolysis cycle. If the disruption in the normal pattern of predominately nocturnal feeding is the cause of the overeating, then it should be expected that when VMH-lesioned rats become obese and their food intakes return to normal levels, that the disruption should disappear. Food intake in obese VMH-lesioned rats is only slightly higher than it is in normal rats, but it is distributed equally between light and dark phases (Becker and Kissileff, 1974; Kakolewski et al., 1971). This suggests that the disruption in the pattern of predominately nocturnal feeding and the hyperphagia are independent. It is also possible, however, that the VMH lesion causes a permanent disruption in the lipostatic mechanism proposed by LeMagnen et al., (1973) and that the reduction in food intake in obese VMH-lesioned rat results from some other compensatory change in the

animal as it gains weight which is unrelated to the lipostatic mechanism.

There is other evidence consistent with the notion that the disruption in the nychthemeral periodicity of feeding and the hyperphagia are independent. Bernardis (1973) has recently produced a disruption in the diurnal distribution of feeding without hyperphagia in weanling rats with a discrete lesion in the dorsomedial nucleus. In addition, the observation in the previous experiment that lactating rats retain nychthemeral periodicity of feeding while eating approximately the same amount as VMH-lesioned rats (with the exception of the 16th-18th days of lactation with a solid diet) demonstrates that rats can overeat without disruption in the normal distribution of light-dark intake. Finally Teitelbaum's (1955) study of the motivational aspects of the VMH syndrome showed that at a low fixed ratio schedule of reinforcement VMH-lesioned rats ate more than normals during a 12-hour period following 12 hours of food deprivation. This experiment did not directly test the hypothesis that overeating is caused by the loss of inhibition on eating during the 12-hour light or lipolytic phase although it tends not to support this hypothesis.

Therefore no direct test has been made of the hypothesis that hyperphagia following VMH lesions is caused by the loss of the lipolytic, light phase inhibition of feeding. It follows that if this is the only cause of the hyperphagia, that VMH-lesioned rats should not overeat if denied access to food during the light phase. In the present experiment in order to test this hypothesis a group of VMH-lesioned rats were prevented from feeding during the light phase to determine if hyperphagia and excessive weight gain could still occur. To control for

the restriction to 12 hours of access, another group of rats was restricted to feeding during the dark.

Since VMH lesions often produce finickiness as well as hyperphagia (Corbit & Stellar, 1964; Graff & Stellar, 1962; Teitelbaum, 1955) and since it has been reported that restricting food in VMH-lesioned rats reduces intake when they are fed dry food (Smith, Salisbury,& Weinberg, 1961) the present experiment was undertaken with three different diets; a milk diet (Becker & Kissileff, 1974), laboratory chow pellets, and a solid high fat diet (Corbit & Stellar, 1964).

In order to be certain that any conclusions reached would not be restricted to a particular body weight or phase of weight gain following VMH lesions, food intakes of the animals in the chow and high fat diet groups were studied immediately after the lesions and again 30-45 days after the lesions when the animals were obese.

Method

Animals

Thirty-nine female rats of the Wistar strain were placed into two groups equated for mean body weight. Twenty of these rats had chronic platinum electrodes (see surgical procedures - General Methods) implanted in the VMH. Six rats had sham operations (see sham implants - General Methods). The other 13 animals were unoperated controls. The intake and its daily distribution were not significantly different on the milk diet (see Table 16) and therefore all animals except two on both the high fat and chow diets were unoperated controls. The data from both the sham operated and unoperated controls have been pooled and will be referred to in the text as "nonlesioned"rats.

Table 16. Food Intake in Sham-Lesioned and Unoperated Controls, Milk Diet

	Before Restriction			Following Restriction	
	Intake (ml)	Percent Intake, light phase	Restriction Intake (ml)	Intake (ml)	Percent Intake, light phase
Sham implant N=4	26.30	19.6	15.15	22.25	21.0
Unoperated control N=4	27.61	25.0	16.95	23.35	21.5
U[1]	4	7	7	5	7
p[1]	.171	.443	.443	.243	.443

1. Rows U and p are the values of U in the Mann Whitney U Test (two-tailed) and the probability that sham implant and controls are the same.

Since animals on different diets gain weight at different rates (Corbit & Stellar, 1964), and since the animals in this experiment were on different diets for the first 30-50 days following the lesion after which they were all placed on laboratory chow (see Sequence of Procedures) total weight gain in the 60 days following the lesion was used in assessing the effectiveness of the VMH lesion. Body weight gains in 60 days following the lesion clustered into three distinct groups with less than 20 g difference within each of the two lowest groups and separated from one another by a difference of 30 g. These separations were the basis of a three group classification of lesioned animals by weight gain. Animals were classified as non-obese (weight gain of less than 113 g which was 10 g higher than the maximum gain in the nonlesioned rats), moderately obese (weight gain between 150 g and 180 g) and obese (weight gain greater than 210 g to a maximum of 370 g). Using this classification 12 rats were classified as obese, four as moderately obese and two as non-obese (see Table 17). The obese and moderately obese rats when considered together will be referred to in the text as "effectively-lesioned" rats. The two other lesioned rats died of unexplained causes on the 10th and 20th days following the lesion and therefore their data are not included.

Housing

During the course of the experiment as well as during the period when weight gain data were collected, all animals were housed individually in mesh bottom cages measuring 24.5 x 18 x 18 cm. The liquid diet was made available from Richter tubes graduated to the ml and clamped to the

Table 17. Body Weight Parameters

Animal	Access Period	Pre-Lesion Rate of Wt. Gain (g/day)	Wt. at Lesion (g)	Number of Days Post Lesion to 2nd Restric.	Pre-2nd Restric. Rate of Wt. Gain (g/day)	Wt. at 2nd Restric. (g)	60-Day Wt. Gain (g)
Milk Diet							
			Obese				
LC-2	D(dark)	3.2	307				300
LC-13	L(light)	5.2	345				211
Mean		4.2	341				255
			Moderately Obese				
LC-4	L	2.8	334				164
LC-1	D	3.0	317				150
Mean		2.9	325				157
			Nonobese				
LC-3	D	2.0	305				93
			Nonlesioned				
LC-9	L	3.8	350				56
LC-12	L	2.4	316				52
LC-10	D	5.2	342				52
LC-11	D	3.4	309				36
LC-6	L	2.6	306				20
LC-7	D	1.4	289				18
LC-5	L	5.0	361				-8
LC-8	D	3.0	270				-9
Mean		3.4	318				27

Table 17 continued

Chow Diet				Obese			
LC-28	D	0.6	262	35	3.4	462	269
LC-14	D	1.2	237	65	4.6	585	247
LC-20	L	-0.2	379	65	0.4	531	238
LC-32	L	3.5	264	35	0.2	447	232
Mean		1.3	260		2.6	506	256
				Moderately Obese			
LC-26	D	2.6	437	35	-1.4	621	178
LC-24	L	2.2	274	65	-1.6	462	172
LC-16	D	-2.8	277	65	2.2	450	156
Mean		0.7	329		-0.3	511	167
				Nonobese			
LC-18	L	1.0	373	65	-1.2	480	111
				Nonlesioned			
LC-25	D	3.6	275	35	0.3	352	102
LC-22	L	1.4	267	65	0.8	298	49
LC-17	L	-1.4	327	65	-1.2	357	44
LC-19	L	1.4	387	65	-1.0	410	34
LC-27	L	0.3	276	35	0.8	270	31
LC-15	D	-0.8	326	65	0.6	351	19
LC-21	D	0.0	315	65	-0.8	331	-11
Mean		0.9	310		-0.1	338	38
HF Diet				Obese			
LC-36	D	2.6	231	30	2.2	565	397
LC-34	D	4.0	261	30	3.7	583	384
LC-38	L	5.0	255	30	-3.0	561	355
LC-40	L	4.6	288	30	-4.2	580	326

Table 17 continued

LC-44	D	1.4	246	23	7.3	465	255
Mean		3.5	256		1.2	550	343
				Nonlesioned			
LC-35	D	2.0	251	30	0.7	303	68
LC-39	L	3.6	291	24	1.5	316	37
LC-37	L	0.8	251	30	0.3	278	35
LC-41	D	5.4	258	23	-0.5	280	28
Mean		3.0	260		0.5	294	42

front of the cage. Laboratory chow pellets were placed on the floor of the cage. The high fat diet was placed in a jar 5.1 cm high and 5.1 cm in diameter and secured inside the cage to its front.

Diets

The liquid diet consisted of sweetened condensed milk diluted by volume three parts milk with one part tap water (see diet - General Methods). The high fat (HF) diet used in this experiment consisted of 33.3 percent (by weight) hydrogenated vegetable fat (Crisco) and 67.7 percent laboratory chow powder (Purina). The caloric density of this diet was 5.83 Kcal/g (based on manufacturer's specifications). The caloric density of laboratory chow pellets (Purina) was 4.25 Kcal/g (based on manufacturer's specifications).

Sequence of Procedures

Rats were adapted to the milk diet for 10 days and to the HF diet for 2 days. Since rats in the chow group were maintained on chow no adaptation period was required. Then body weight, and food intake (corrected for spillage) and water intake were recorded twice daily just before the lights were turned off (7 p.m. local time) and just after the lights were turned on (7 a.m. local time). After 5 days, both VMH-implanted and nonlesioned groups were divided into subgroups, each equated for body weight (see Table 17). One implanted and one nonlesioned subgroup was treated as follows in the morning (7 a.m.) and the other two subgroups were treated as follows in the evening (7 p.m.). At the usual time of collecting data, either 7 a.m. or 7 p.m., animals were removed from their cages and lightly anesthetized with ether. Implanted animals were lesioned (see lesioning - General Methods) and nonlesioned animals were etherized for approximately the time required for lesioning the experimental group (about 5 minutes). All rats were then immediately

returned to their cages and given the experimental diet for the next 12 hours.

Access to food was then restricted to alternate 12-hour periods for the next 4 - 5 days. For one group of VMH-lesioned (N=8) and nonlesioned rats (N=10) referred to from now as the light access group (LA) feeding was limited to the period when the lights were on (7 a.m. - 7 p.m.). For the other group of VMH-lesioned rats (N=10) and nonlesioned rats (N=9) referred to as the dark access group (DA) feeding was restricted to the dark period (7 p.m. - 7 a.m.).

Following restriction all animals were returned to 24-hour food access. Body weight and food and water intakes were measured twice daily just before the lights were turned off and just after the lights were turned on for 4-5 additional days (restoration period).

The rats fed milk (N=13) were maintained on the milk diet for 20 days after the restoration period ended and their body weights were recorded on the 30th days after lesioning. They were then placed on laboratory chow pellets for 30 additional days to determine if the excess weight gains were due to the high palatability of the diet to which VMH-lesioned rats are extremely sensitive (Corbit & Stellar, 1964). The animals on the chow and HF diets (both VMH-lesioned and nonlesioned) had ad libitum access to their diets for an additional 20 to 50 and 10 to 15 days respectively when body weight of the VMH-lesioned rats was elevated from 150 to 305 g (see Table 17). Food intake in both VMH-lesioned and nonlesioned rats were then again measured every 12 hours for 5 days.*

*Only 3 days for LC-41 and LC-44.

LA and DA animals were then restricted to feeding every 12 hours per day during the light and dark phases respectively for the next 5 days. Then food was returned, ad libitum, and intakes were recorded every 12 hours for the next 5 days. Animals on the HF diet were then given laboratory chow pellets, ad libitum, until 60 days from the lesion had passed. All rats were weighed on the 60th day after the lesion. Between the 60th and 85th days post lesion animals were sacrificed with an overdose of Nembutal, and perfused with 10 percent formalin in isotonic saline (see histology - General Methods).

Data Analysis

In order to isolate the immediate effects of the lesions and the limitation to only 12 hours of access, intake during restriction was the amount consumed in the 12 hours immediately following each restriction period. (The amount consumed in the first 12 hours after lesioning was considered separately). The percentage of light phase intake was calculated by dividing the amount consumed during the light phase by the total daily (24-hour) intake and multiplying the quotient by 100. The percentage change was calculated by subtracting the original value from the new value and dividing by the original value (quantity multiplied by 100). Where a mean percentage change is given, it is simply the arithmetic mean of the percentage changes occurring in individual animals.

Body weight changes were calculated in the following manner in order to assure that the normal daily fluctuations in body weight did not

affect results. Body weight gain before the lesion or anesthization was the body weight at the time of the lesion or anesthization subtracted from the body weight 5 days earlier at the corresponding time of day. Body weight gain during restriction was the difference between body weight at the end of the last restriction period and body weight at the end of the last light phase period before restriction. The reason for this procedure was to compare body weight gains at points of time when differences in stomach fullness would contribute least to weight gain. For example, comparing body weight of a VMH-lesioned rat after 12 hours of eating to its pre-lesion body weight at the end of the light phase would greatly overinflate the weight gain because in the pre-lesion condition food intake during the light phase period was minimal. Body weight gain following restriction was the difference between weight 12 hours after the last restriction and body weight 5 days later at the corresponding time of day.

The statistical tests employed in this experiment were the Mann Whitney U Test (two-tailed, Mendenhall, 1971) and an extension of the two-factor analysis of variance with repeated measures on each factor (personal communication, Michels) presented in Winer (1971). A detailed explanation of this method is provided in Appendix A.

Results

All effectively lesioned rats displayed hyperphagia and excessive body weight gains on all experimental diets despite restriction to only 12 hours of food access in the five days following lesioning. Neither food intake nor the rate of body weight gain during restriction differed between LA and DA animals. Nonlesioned rats consumed less than normal amounts of food during restriction and hence did not gain weight at a normal rate during the restriction.

When restricted to 12 hours of access to food after becoming obese, VMH-lesioned rats ate less than they had eaten in 24 hours during the five days prior to the restriction and lost body weight.

Distribution of Intake Before Lesion and Restriction

Before the lesion or anesthetization there were no significant differences between VMH-implanted and nonlesioned rats in mean daily light phase intake ($p=.404$, $U=14$, $n_1=4$, $n_2=8$ for milk; $p=.095$, $U=16$, $n_1=8$, $n_2=7$ for chow; $p=.143$, $U=5$, $n_1=5$, $n_2=4$ for HF), mean daily dark phase intake ($p>.50$, $U=17$, $n_1=4$, $n_2=8$ for milk; $p>.50$, $U=23$, $n_1=8$, $n_2=7$ for chow; $p=.143$, $U=4.5$, $n_1=5$, $n_2=4$ for HF), or mean total daily intake ($p=.467$, $U=15$, $n_1=4$, $n_2=8$ for milk; $p=.198$, $U=20$, $n_1=8$, $n_2=7$ for chow; $p=.452$, $U=9$, $n_1=5$, $n_2=4$ for HF; Mann Whitney U Test, two-tailed), on each of the three diets. This demonstrates that the VMH-implanted and nonlesioned rats were not statistically different on the critical dependent variables (intakes) before the independent variable (namely the lesion) was introduced. There were also no significant differences between the whole DA group (nonlesioned and VMH-implanted animals)

and the whole LA group (nonlesioned and VMH-implanted) in either light phase intake ($p=.314$, $U=17$, $n_1=6$, $n_2=7$ for milk; $p=.523$, $U=28.5$, $n_1=7$, $n_2=8$ for chow; $p=.143$, $U=5$, $n_1=5$, $n_2=4$ for HF; Mann Whitney U Test, two-tailed) dark phase intake ($p=.223$, $U=15$, $n_1=6$, $n_2=7$ for milk; $p=.221$, $U=22.5$, $n_1=7$, $n_2=8$ for chow; $p=.206$, $U=5.5$, $n_1=5$, $n_2=4$ for HF; Mann Whitney U Test, two-tailed) or total daily intake ($p=.183$, $U=14$, $n_1=6$, $n_2=7$ for milk; $p=.389$, $U=24$, $n_1=7$, $n_2=8$ for chow; $p=.500$, $U=12$, $n_1=5$, $n_2=4$ for HF; Mann Whitney U Test, two-tailed)on each of the three diets. This is important because it demonstrates that before food restriction there were no differences in mean daily intakes between the two groups of animals to be restricted.

The feeding behavior of both VMH-implanted and nonlesioned animals was predominately nocturnal. The percentages of light phase intake for all of the animals on each of the diets were a mean of 23.5 (Range=9.6-3.90) for milk, 23.1 (R=6.3-42.0) for chow, and 13.7 (R=0.0-26.0) for high fat. The lower percentage of light phase intake of the animals on HF just bordered on being significantly different from the percentage of light phase intake of the animals on milk ($p>.05$, $U=35$, $n_1=13$, $n_2=0$, Mann Whitney U Test, two-tailed). The critical value of U for significance ($p<.05$) was 34. There were no significant differences in the percentage of light phase intake between rats on chow and rats on HF ($p>.05$, $U=36$, $n_1=13$, $n_2=9$, Mann Whitney U Test, two-tailed) and between rats on milk and rats on chow ($p>.05$, $U=81$, $n_1=13$, $n_2=15$, Mann Whitney U Test, two-tailed).

Response to Lesion in First 12 Hours: Effectively-Lesioned Rats

All effectively lesioned rats on each diet and lighting condition were eating within a half-hour following the lesion. Within the first 12 hours after the lesion they had all eaten more than any of the merely anesthetized rats had eaten following anesthetization (cf. Figures 17, 18, and 19, 20). Also, effectively lesioned rats in all dietary and lighting conditions except the LA rats fed chow, ate more in the first 12 h after the lesion than they had eaten in any 24 h period before they had been lesioned. In fact, this early increase in intake turns out to be the most reliable procedure of determining effectively lesioned animals as judged by later obesity. Food intake of the LA rats fed chow was less in the first 12 h following lesions than the mean 24 h intake of the preceding 5 days but was greater than either the light phase or dark phase intake before lesions. Although intake across light and dietary conditions increased from 9 to 95 percent there were no other significant differences among the mean percentage increases across either light or dietary conditions other than the one already noted between LA and DA rats fed chow.

Response to Restriction

In all effectively lesioned rats food intake during the days of restricted access remained elevated (see Figures 17 and 18, 12 hour access). There were no significant differences in food intakes between DA and LA rats on any of the diets during the periods of restricted access (p cannot be calculated, n too small for milk; $p=.314$, $U=4$, $n_1=3$, $n_2=4$ for chow; $p=.20$, $U=1$, $n_1=2$, $n_2=3$ for HF; Mann

Figure 17. Means and ranges of daily intakes of effectively VMH-lesioned DA rats consuming milk (top), chow (middle), and HF (bottom) diets. The wide bars on the left indicate mean daily intake for the 5 days immediately preceding the VMH lesion. The darkened portions of these bars indicate mean daily intake for the 5 days immediately preceding the lesion during the dark phase and the light portions of mean daily intake during the light phase. The darkened bars in the center of the figure indicate mean daily intake during the first 12 hours immediately following the lesion and intake when access to food was restricted to the dark phase only. The bars on the right side of the figure indicate mean daily intake for the 4-5 days following the restriction. Their darkened portions indicates mean daily dark phase intake and their light portions mean daily light phase intake. The vertical lines extending above and below each bar on the figure represent the ranges of mean daily intakes for each animal in that period.

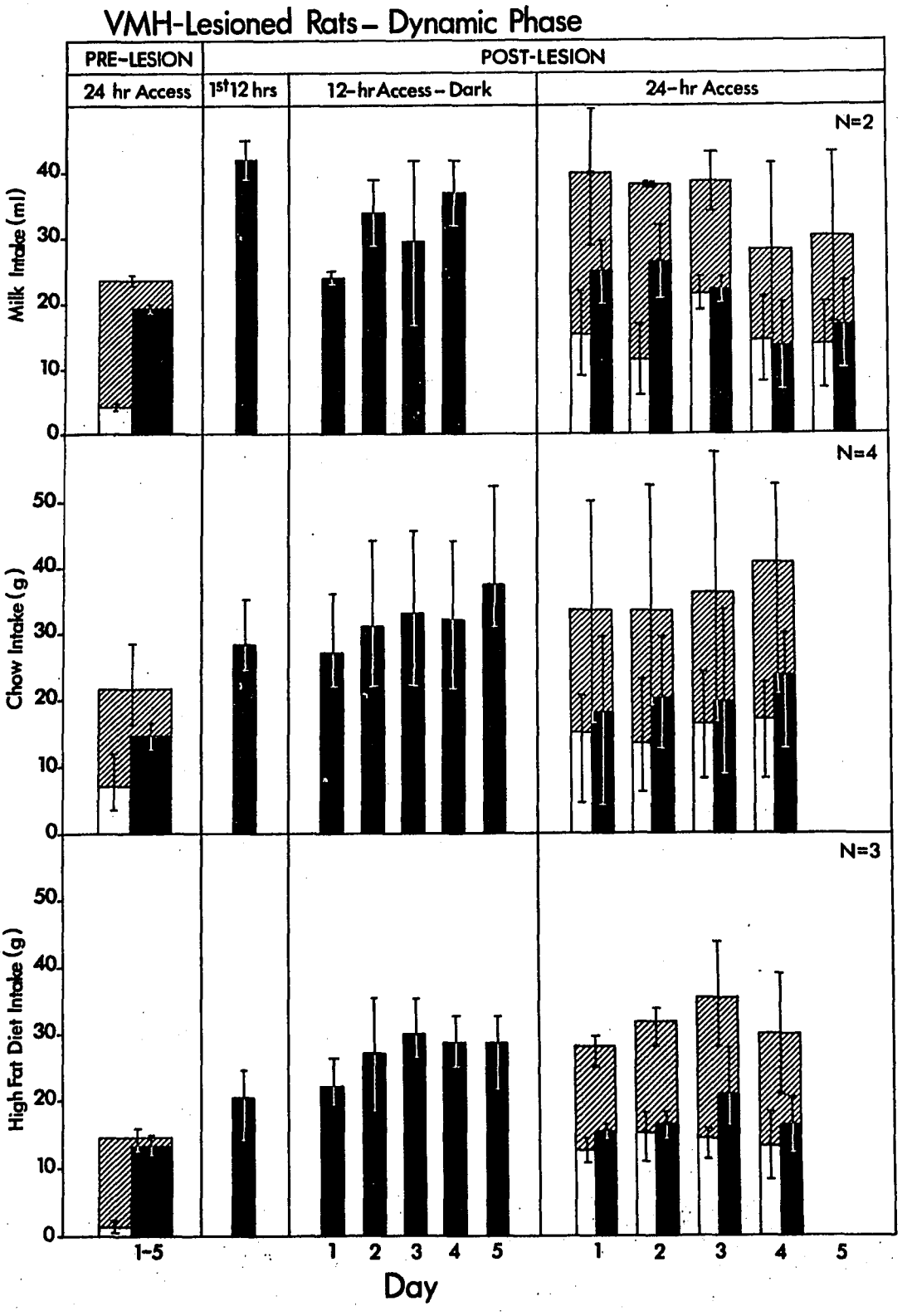
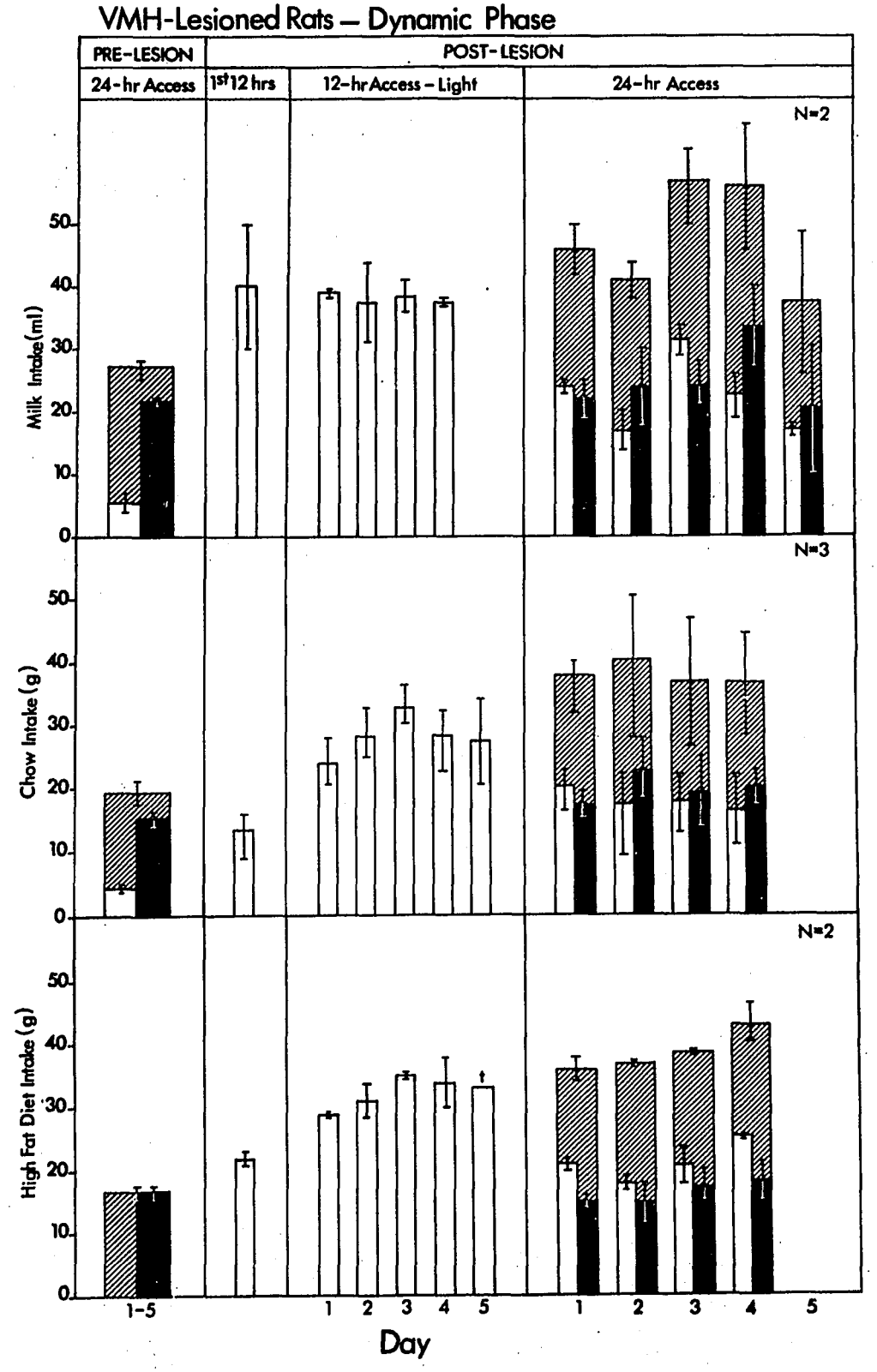

VMH-Lesioned Rats – Dynamic Phase
PRE-LESION
POST-LESION
24 hr Access
1st 12 hrs
12-hr Access – Dark
24-hr Access
N=2
N=4
N=3
Milk Intake (ml)
Chow Intake (g)
High Fat Diet Intake (g)
0
10
20
30
40
50
1-5
1
2
3
4
5
Day

Figure 18. Means and ranges of daily intakes of the effectively VMH-lesioned LA rats consuming milk (top), chow (middle), and HF (bottom) diets. The wide bars on the left indicate mean daily intake for the 5 days immediately preceding the lesion during the dark phase and the light portions mean daily intake during the light phase. The light bars in the center of the figure indicate mean intake during the first 12 hours following the lesion and mean daily intake when access to food was restricted to the light phase. The bars on the right side of the figure indicate mean daily intake for the 4-5 days following the restrictio-. Their darkened portions indicate mean daily dark phase intake and their light portions mean daily light phase intake. The vertical lines extending above and below each bar on the figure represent the ranges of the mean daily intakes from each rat for that period.


VMH-Lesioned Rats – Dynamic Phase
PRE-LESION
POST-LESION
24-hr Access
1st 12 hrs
12-hr Access – Light
24-hr Access
N=2
N=3
N=2
Milk Intake (ml)
Chow Intake (g)
High Fat Diet Intake (g)
0
10
20
30
40
50
1-5
1
2
3
4
5
Day

Whitney U Test, two-tailed). Therefore in comparing the percentage increase in food intake from pre-lesioned levels during restricted access, data from LA and DA rats have been pooled. The only significant difference in percentage increase in intake was between the rats fed HF and rats fed milk (no overlap in ranges). Food intake was elevated over pre-lesion levels by different percentages among dietary conditions. Rats fed the HF diet showed the greatest percentage increase in food intake from pre-lesion levels (Mean=92.7, R=67.3-104.8). Rat fed chow had the next greatest percentage increase (M=46.8, R=13.4-90.7) while rats fed milk had the least increase (M=37.1, R=10.0-55.5). In addition, all effectively-lesioned rats on each diet ate more food than any of the non-lesioned animals on the corresponding diet, but the percentage differences varied with the diet and were 149.7, 97.9, and 86.0 for rats fed HF, milk, and chow respectively.

Although food intake remained elevated during the restriction period it did not remain constant. Food intakes of the animals fed the two solid diets (chow and HF) gradually increased during restriction (see Figures 17 and 18, 12-hour access). Food intake of the DA animals fed milk dropped slightly and was more variable than food intake of the LA animals fed milk. Food intake of the LA animals fed milk showed no systematic departure from its value after the lesions were made (see Figures 17 and 18, 12-hour access).

Return to 24-hour Access

Upon return to 24-hour access each lesioned animal except one (LC 20)

increased its food intake abruptly to a new plateau, although the degree of increase varied across diets, but not across different conditions of prior access to food. Food intake increased a mean percentage of 36.9 (R=decrease of 2.0, increase of 62.6) in rats fed chow, 15.8 (R=5.4-22.4) in animals fed HF, and 12.3 (R=5.1-31.7) in rats on milk. These differences were statistically significant (see Tables 18, 19, and 20). Therefore this experiment demonstrates that the hyperphagia in VMH-lesioned rats is reduced, but not abolished by restriction to 12 hours of access to food. However the percentage reduction in intake in the VMH-lesioned rats resulting from the limitation to 12 hours of access to food was not different from the percentage reduction in intake occurring in the nonlesioned rats on each of the three diets ($p>.50$, $U=21$, $n_1=4$, $n_2=8$ for milk; $p>.50$, $U=23.5$, $n_1=7$, $n_2=7$ for chow; $p=.548$, $U=10$, $n_1=4$, $n_2=5$ for HF; Mann Whitney U Test, two-tailed). Therefore the effect on food intake of restriction to 12 hours of access is not different in dynamic VMH-lesioned rats and non-lesioned rats.

In addition when returned to 24 hour access, intake in all VMH-lesioned animals became immediately distributed equally between the light and dark phases (see Figures 17 and 18, 24-hour access post lesion). This equal distribution of intake was accomplished by a reduction in dark phase intake for DA animals and light phase intake for LA rats.

Moderately Obese and Nonobese Rats

Moderately obese rats (LC-1, -4, -16, -24) responded in all cases like the other rats in their groups but their intakes with one exception were lower than the intakes of the obese rats in their groups at each point in the experiment. The exception was LC-4 whose intake in the first 12 hours after the lesion and during restriction was lower than its obese partner (LC-13) but whose intake in the post restriction period was higher. Of the two nonobese rats (LC-3 and LC-18), LC-18's intake was not outside the range of intakes of the nonlesioned rats at any point in the experiment. The other nonobese rat (LC-3) was finicky. It responded like the other lesioned animals in its group, but when returned to chow lost weight precipitously unlike the other obese animals which maintained or increased their weights (see Table 17).

Nonlesioned Rats

Food intake of the nonlesioned rats during the 12 h period following anesthesia was not significantly different from the food intakes before anesthesia during either the light phase for the LA animals or the dark phase for the DA animals ($p>.05$, $U=49$, $n_1=10$, $n_2=10$ for LA animals; $p>.05$, $U=30$, $n_1=9$ for DA animals, Mann Whitney U Test, two-tailed. cf. Figures 19 and 20 pre-anesthesia and 12-hours post anesthesia).

Food intakes of the DA and LA rats differed initially during restriction (see Figures 19 and 20). DA animals on all three diets continued to eat during the restriction the same amount that they had eaten during the first 12 hours following anesthesia (see Figure 19) which was also the same as the intake before anesthesia during the

Figure 19. Means and ranges of daily intakes of the nonlesioned DA rats consuming milk (top), chow (middle), and HF (bottom) diets. The wide bars on the left indicate mean daily intake for the 5 days immediately preceding anesthetization. The darkened portions of these bars indicate mean daily dark phase intake and the light portions mean daily light phase intake for the 5 days immediately preceding anesthetization. The dark bars in the center of the figure indicate mean daily intake during the 12 hours following anesthetization and mean daily intake when access to food was restricted to the dark phase. The bars on the right side of the figure indicate mean daily intake for the 4-5 days following restriction. Their darkened portions indicate mean daily dark phase intake and their light portions mean daily light phase intake. The lines above and below each bar on the figure represent the ranges of mean intakes from each animal for that period.

/ Data collected on two animals only during the light phase period. Total and dark phase intake are the data from only one rat.

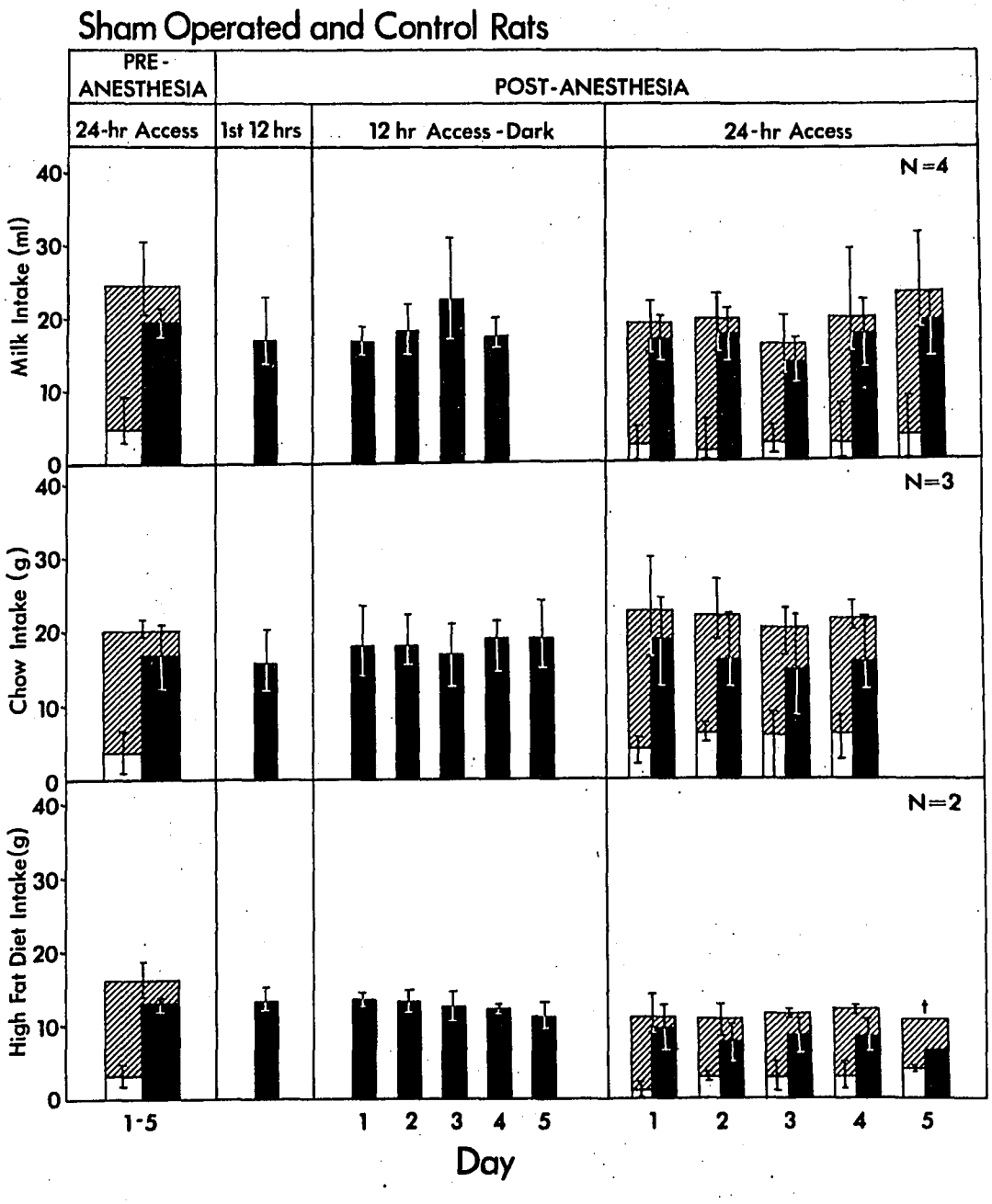
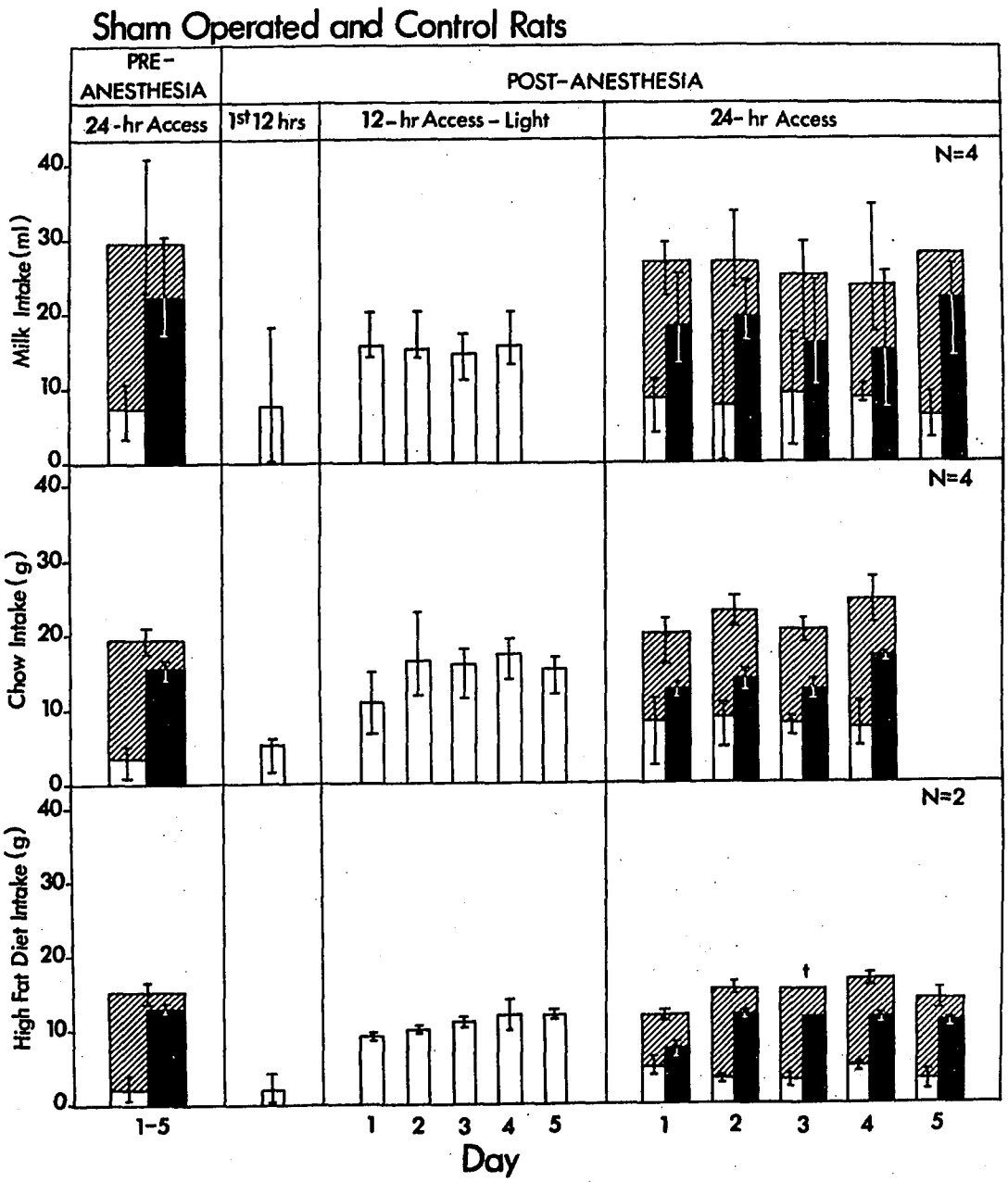

Sham Operated and Control Rats
PRE-ANESTHESIA
POST-ANESTHESIA
24-hr Access
1st 12 hrs
12 hr Access - Dark
24-hr Access
N=4
N=3
N=2
Milk Intake (ml)
Chow Intake (g)
High Fat Diet Intake(g)
0
10
20
30
40
1-5
1
2
3
4
5
Day

Figure 20. Means and ranges of daily intakes of the nonlesioned LA rats consuming milk (top), chow (middle), and HF (bottom) diets. The wide bars on the left indicate mean daily intake for the 5 days immediately preceding anesthetization. The darkened portions of these bars indicate mean daily intake during the dark phase and the light portions mean daily intake during the light phase for the 5 days immediately preceding anesthetization. The light bars in the center of the figure show mean daily intake during the 12 hours immediately following anesthetization and mean daily intake when access was restricted to the light phase. The bars on the right side of the figure indicate mean daily intake for 4-5 days following restriction. Their darkened portions indicate mean daily dark phase intake and their light portions mean daily light phase intake. The lines above and below each bar on the figure represent the ranges of mean intakes from each animal for that period.


Sham Operated and Control Rats
PRE-ANESTHESIA
POST-ANESTHESIA
24-hr Access
1st 12 hrs
12-hr Access - Light
24-hr Access
N=4
N=4
N=2
Milk Intake (ml)
Chow Intake (g)
High Fat Diet Intake (g)
0
10
20
30
40
1-5
1
2
3
4
5
Day

dark phase (cf. Figure 19, 24-hour access, pre-anesthesia and 12-hour access post anesthesia). Food intake of LA animals on chow and the HF diet was initially depressed during restriction but gradually rose and by the second or third day of restriction was no different from food intake of the DA animals on the corresponding diet (cf. Figures 19 and 20). The LA animals fed milk were exceptional, since their initially low intakes remained low through the restriction period (see Figure 20). The mean intake of rats fed milk was lower for LA than for DA (cf. Figures 19 and 20), but this difference was not significant ($p=.057$, $U=2$, $n_1=4$, $n_2=4$ Mann Whitney U Test, two-tailed). There was, however, a statistically larger drop in the intakes of the LA rats than of the DA rats fed milk ($p=.029$, $U=1$, $n_1=4$, $n_2=4$, Mann Whitney U Test, two-tailed). Mean daily food intakes on each day of restriction were always slightly less than mean daily total 24-hour intakes before restriction for both DA and LA rats on all three diets (see Figures 19 and 20).

Upon return to 24- hour access, food intake immediately returned to its pre-restriction level, except in DA animals on the high fat diet in which intake remained a mean of 14.6 (R=6.0 more to 42.2 less) percent of its pre-restriction value. The predominantly nocturnal nychthemeral distribution of food intake returned immediately in all rats (both DA and LA on all three diets). However, in LA rats fed chow, intake during the light phase was greater than before restriction (see Figure 20).

Body Weight Changes

Before Restriction. The rate of body weight gain was not significantly different between DA and LA animals ($p=.472$, $U=19$, $n_1=5$, $n_2=8$ for milk; $p=.150$, $U=21$, $n_1=9$, $n_2=7$ for chow; $p-.365$, $U=8$, $n_1=5$, $n_2=4$ for HF; Mann Whitney U Test, two-tailed) or between lesioned and nonlesioned animals before the lesions or anesthetization in rats compared within each dietary condition ($p=.183$, $U=13.5$, $n_1=6$, $n_2=4$ for milk; $p=.223$, $U=21$, $n_1=7$, $n_2=8$ for chow; $p=.305$, $U=9$, $n_1=6$, $n_2=4$ for HF; Mann Whitney U Test, two-tailed; cf. Figures 21, 22, and 24, 25). However, body weight gains differed significantly across dietary conditions. Animals (both VMH-implanted and nonlesioned) fed the HF diet and animals fed milk gained significantly more body weight than the animals fed chow ($p < .02$, $U-24$, $n_1=13$, $n_2=15$ between milk and chow; $p < .02$, $U=18.5$, $n_1=15$, $n_2=9$ between chow and HF; Mann Whitney U Test, two-tailed). There were no significant differences in the rate of body weight gain between animals fed milk and animals on the HF ($p > .05$, $U=59$, $n_1=13$, $n_2=9$, Mann Whitney U Test, two-tailed.

During Restriction. During restriction the rate of body weight gain exceeded the rate of body weight gain before the restriction in all effectively-lesioned rats on all three diets (see Figures 21 and 22). There were no differences in the rate of body weight gain between effectively lesioned DA and LA rats on each of the three diets (p cannot be calculated, n too small for milk; $p = .20$, $U=3$, $n_1=4$, $n_2=3$ for chow; $p=.20$, $U=1$, $n_1=3$, $n_2=2$ for HF; Mann Whitney U Test, two-tailed).

Figure 21. Means and ranges of the body weight changes of the effectively VMH-lesioned DA animals on the three experimental diets. The mean rate of body weight gain for the 5 days immediately preceding the lesion and restriction is shown on the left-hand side of the figure. The mean rate of body weight gain during restriction is shown in the center of the figure, and mean rate of body weight gain for the 5 days immediately following the restriction is shown on the right-hand side of the figure. The lines above and below each bar represent the range of body weight gains in individual animals for that period. For a complete description of how the rate of body weight gain was calculated, see data analysis section.

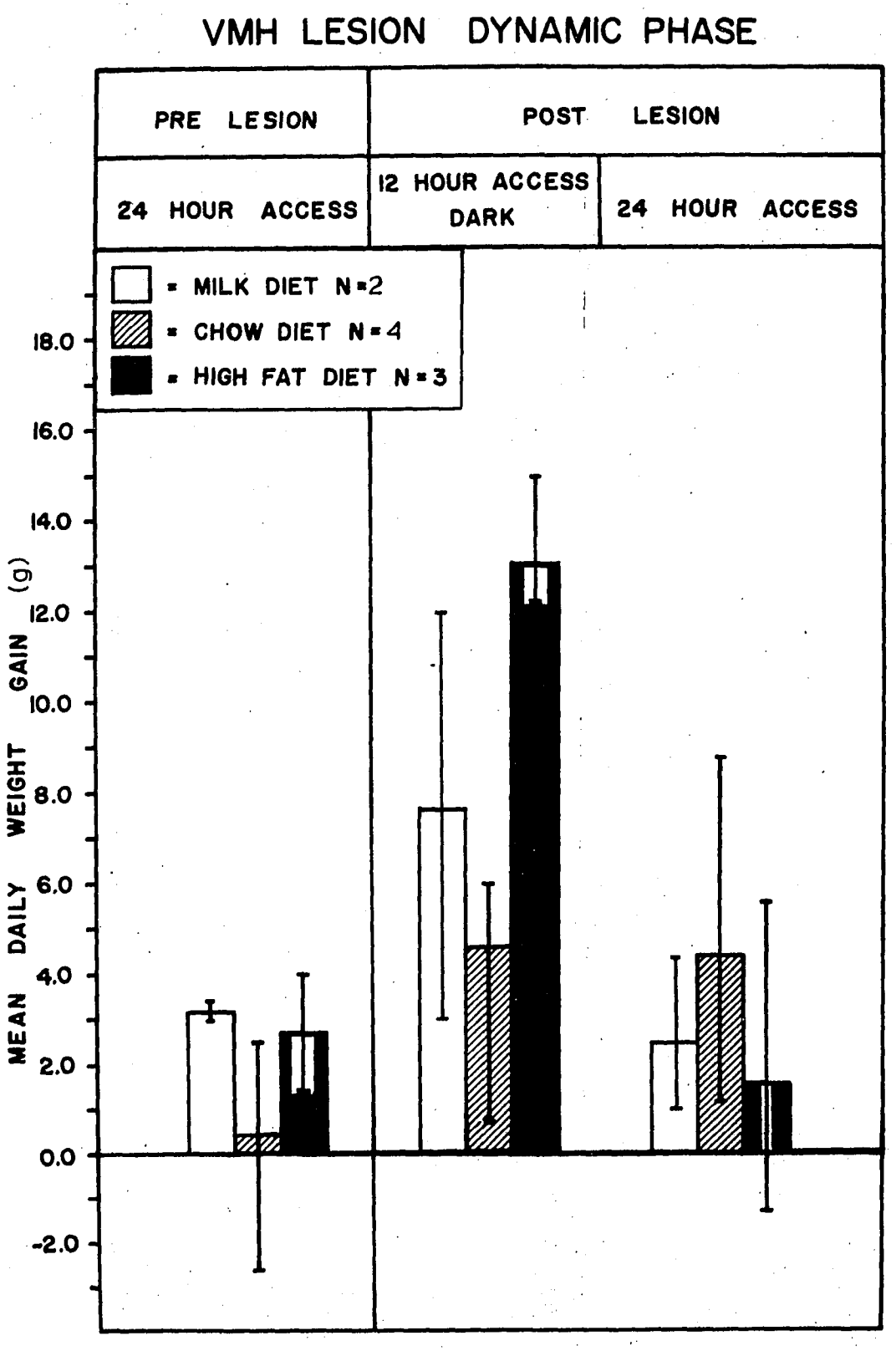
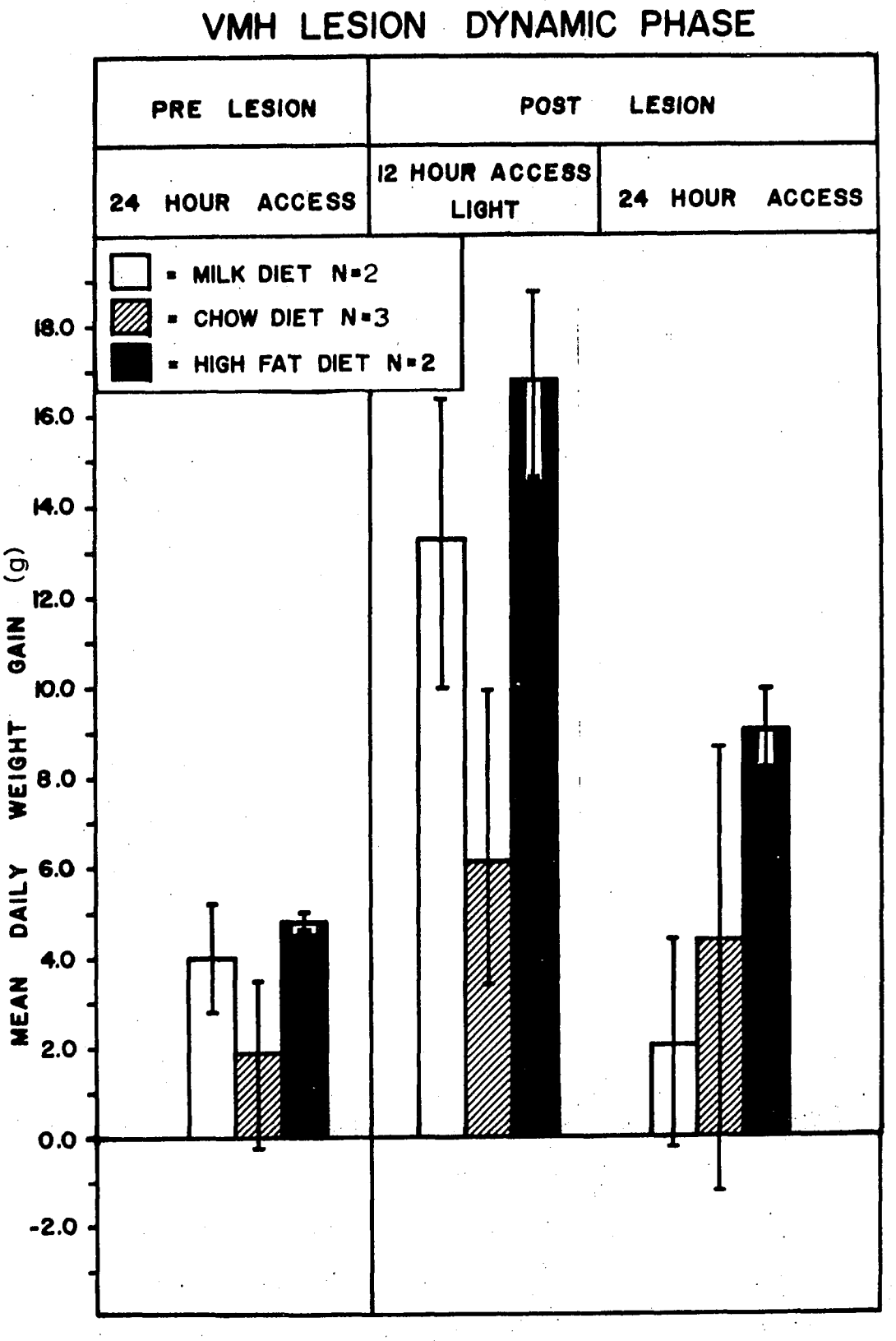

VMH LESION DYNAMIC PHASE
PRE LESION
POST LESION
24 HOUR ACCESS
12 HOUR ACCESS DARK
24 HOUR ACCESS
= MILK DIET N=2
= CHOW DIET N=4
= HIGH FAT DIET N=3
MEAN DAILY WEIGHT GAIN (g)
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
-2.0

Figure 22. Means and ranges of the body weight changes of the effectively VMH-lesioned LA animals on the three experimental diets. The mean rate of body weight gain for the 5 days immediately preceding the lesion and restriction is shown on the left-hand side of the figure. The mean rate of body weight gain during restriction is shown in the center of the figure, and mean rate of body weight gain for the 5 days immediately following restriction is shown on the right-hand side of the figure. The lines above and below each bar represent the range of body weight gains in individual animals for that period. For a complete description of how the rate of body weght gain was calculated, see data analysis section.


VMH LESION DYNAMIC PHASE
PRE LESION
POST LESION
24 HOUR ACCESS
12 HOUR ACCESS LIGHT
24 HOUR ACCESS
= MILK DIET N=2
= CHOW DIET N=3
= HIGH FAT DIET N=2
MEAN DAILY WEIGHT GAIN (g)
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
-2.0

There was no indication that the rate of body weight gain in the five days immediately following a VMH-lesioned rat is retarded by restriction to only 12-hours of access to food. The rate of body weight gain of the obese and moderately obese rats on the milk diet in this study was not significantly different from the rate of body weight gain of the obese and moderately obese lesioned rats allowed 24-hour access to the same diet examined in a previous study (Becker & Kissileff, 1974; $p=.230$, $U=11$, $n_1=4$, $n_2=8$; Mann Whitney U Test-two-tailed; see Figure 23). Both groups of animals are presumed to have equally effective lesions since their total 30-day weight gains were not significantly different ($p=.184$, $U=9.5$, $n_1=4$, $n_2=8$; Mann Whitney U Test, two-tailed; see Figure 23).

In contrast to the lesioned rats, the rate of body weight gain in all nonlesioned rats was reduced during the restriction and most (13 of 19) nonlesioned rats lost body weight during the restriction period. The rate of body weight change during restriction between nonlesioned DA and LA animals was not significantly different on each of the three diets ($p=.171$, $U=6$, $n_1=4$, $n_2=4$ for milk; $p=.50$, $U=4.5$, $n_2=3$, $n_2=4$ for chow; p cannot be calculated, n too small for HF; Mann Whitney U Test, two-tailed, (cf. Figures 24 and 25).

Dietary conditions influenced weight change during restriction in both lesioned and nonlesioned rats. In the effectively-lesioned rats body weight gains of the rats fed milk ($M=10.6$ g per day, $R=3.3$-16.5) and HF ($M=14.6$, $R=12.2$-18.8) were not significantly different ($p=.548$, $U=10$,

Figure 23. Means and ranges of the rates of body weight gains in the 5 days immediately following the VMH-lesion for the 4 effectively lesioned animals fed milk and restricted to 12 hours of access to food and 9 effectively-lesioned (obese and moderately obese) rats from a previous study (Becker & Kissileff, 1974) fed the same milk diet (left side). Means and ranges of the rates total body weight gained in 30 days following the VMH lesion for the 4 effectively lesioned animals fed milk in this study and 9 effectively lesioned (obese and moderately obese) rats from a previous study (Becker & Kissileff, 1974) fed the same diet (right side). The mean is shown by the height of the bar and the range in individual animals is indicated by the lines above and below the bar.

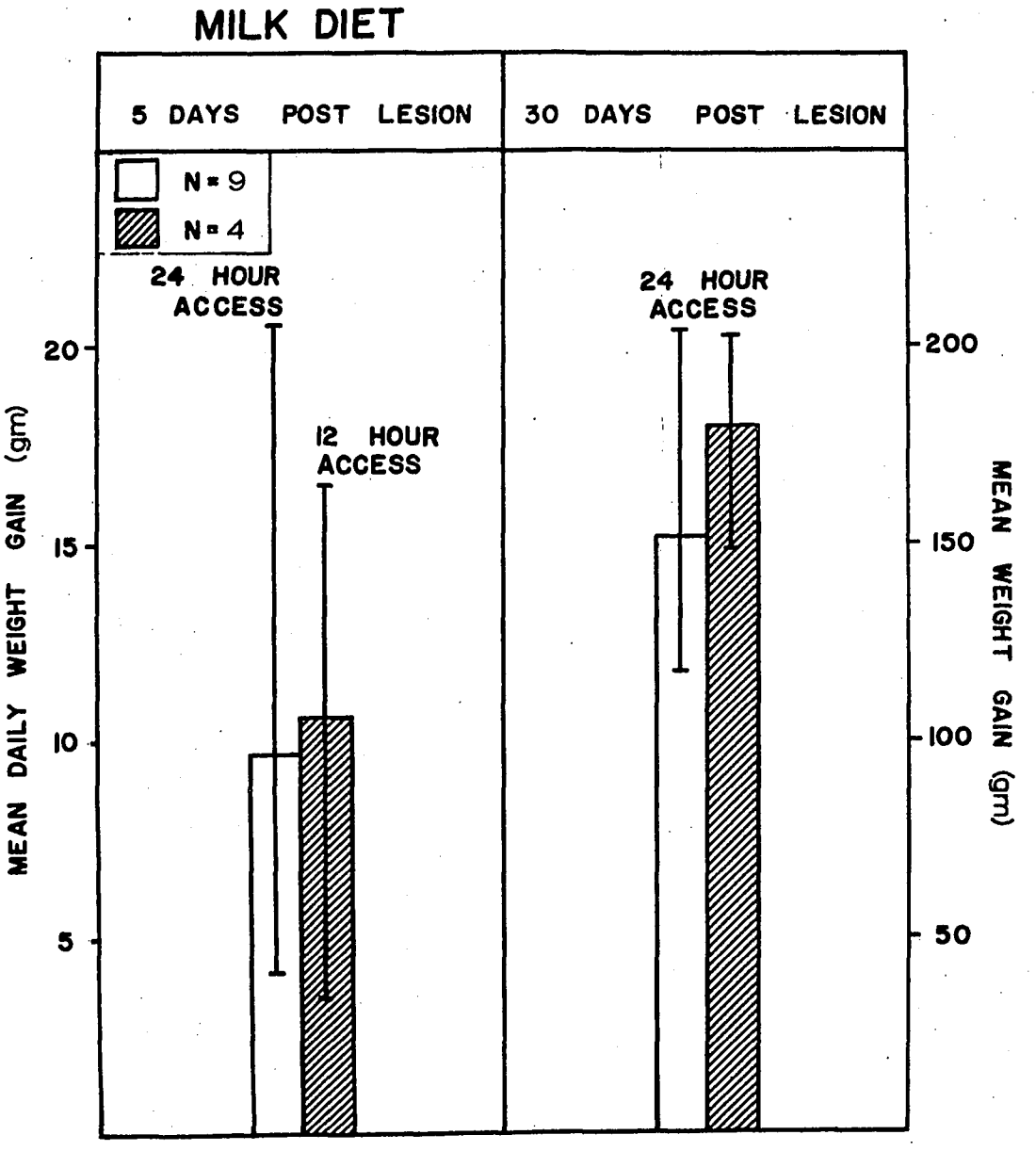

MILK DIET
5 DAYS POST LESION
30 DAYS POST LESION
N = 9
N = 4
24 HOUR ACCESS
12 HOUR ACCESS
24 HOUR ACCESS
MEAN DAILY WEIGHT GAIN (gm)
20
15
10
5
200
150
100
50
MEAN WEIGHT GAIN (gm)

Figure 24. Means and ranges of body weight changes of the non-lesioned DA animals on the three experimental diets. The mean rate of body weight gain for the 5 days immediately preceding anesthetization and restriction is shown on the left-hand side of the figure. The mean rate of body weight change during restriction is shown on the center of the figure, and the mean rate of body weight change for the 5 days immediately following restriction is shown on the right-hand side of the figure. The lines above and below each bar represent the range of body weight changes in individual animals for that period. For a complete description of how the rate of body weight changes was calculated, see data and data analysis section.

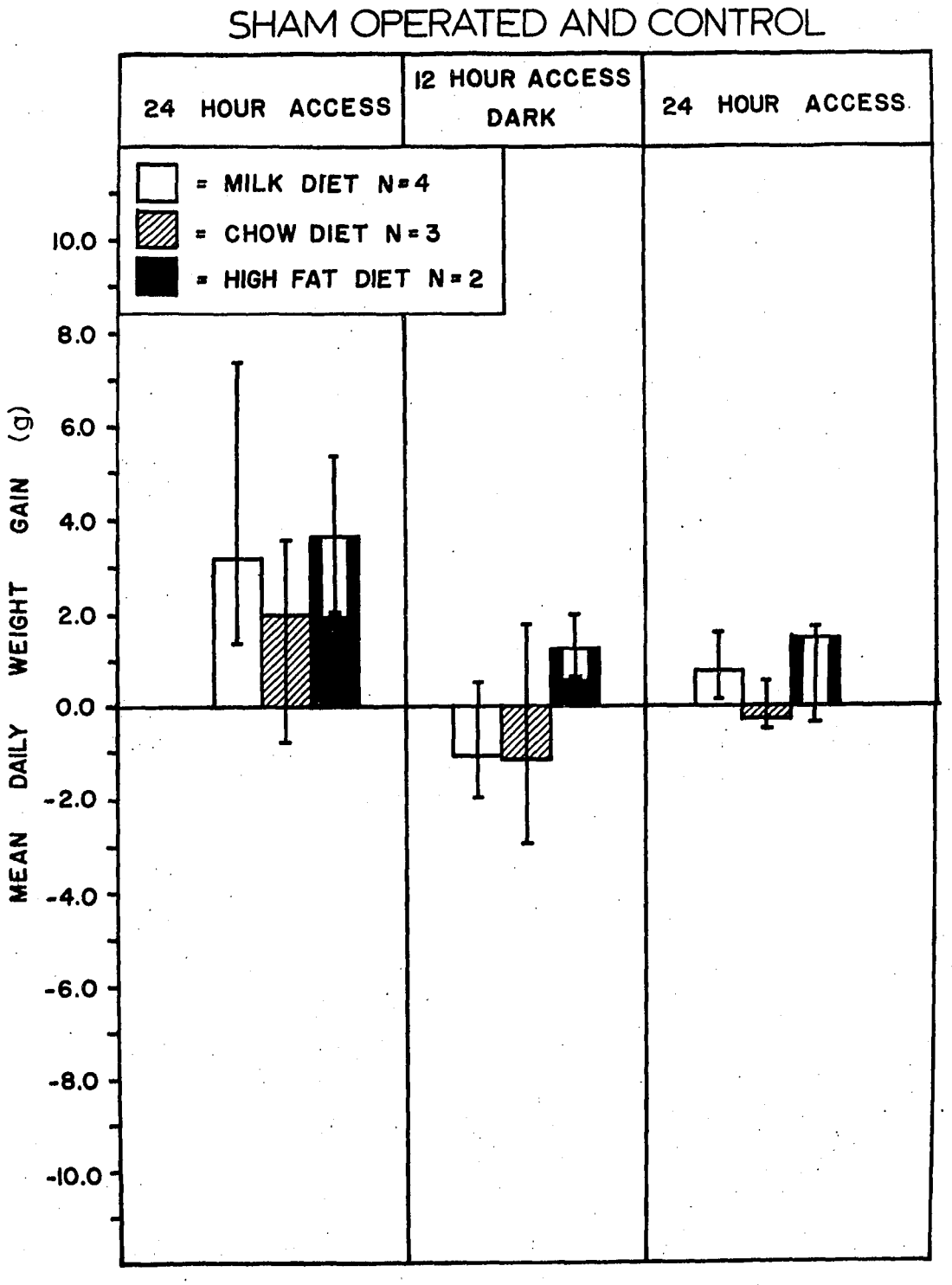
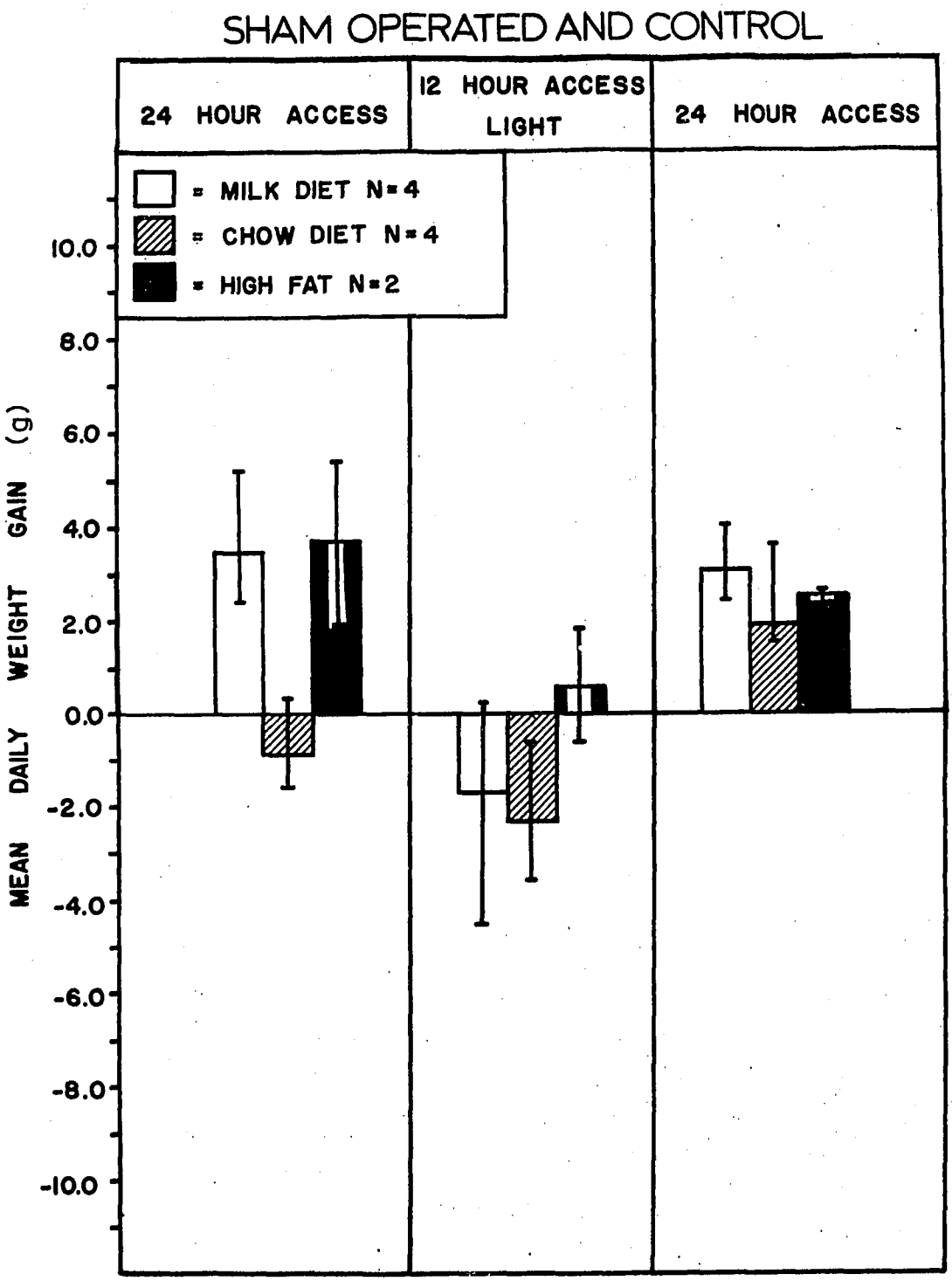

SHAM OPERATED AND CONTROL
24 HOUR ACCESS
12 HOUR ACCESS DARK
24 HOUR ACCESS
= MILK DIET N=4
= CHOW DIET N=3
= HIGH FAT DIET N=2
MEAN DAILY WEIGHT GAIN (g)
10.0
8.0
6.0
4.0
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0

Figure 25. Means and ranges of body weight changes of the non-lesioned LA animals on the three experimental diets. The mean rate of body weight gain for the 5 days immediately preceding anesthetization and restriction is shown on the left-hand side of the figure. The mean rate of body weight change during restriction is shown in the center of the figure, and the mean rate of body weight changes for the 5 days immediately following restriction is shown on the right-hand side of the figure. The lines above and below each represent the range of body weight changes in individual animals for that period. For a complete description of how the rate of body weight changes was calculated, see data analysis section.


SHAM OPERATED AND CONTROL
24 HOUR ACCESS
12 HOUR ACCESS LIGHT
24 HOUR ACCESS
= MILK DIET N=4
= CHOW DIET N=4
= HIGH FAT N=2
MEAN DAILY WEIGHT GAIN (g)
10.0
8.0
6.0
4.0
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0

n_1=4, n_2=5, Mann Whitney U Test, but body weight gain of rats fed chow (M=5.8 g per day, R=0.6-10.0) was significantly less than body weight gain of rats fed HF (no overlap in range of body weight gains). In nonlesioned rats, the body weight change on the HF diet (M=1.0, R=-0.6-2.0) was significantly different from the body weight changes on either milk (M=1.35 g per day, R=-2.5-0.25) or chow (M=1.8 g per day, R=-3.6-1.8) (p=0.014, U=3, n_1=8, n_2=4 between milk and HF; p=.021, U=3, n_1=7, n_2=4 between chow and HF; Mann Whitney U Test, two tailed).

Following restriction. In the 5-day period following the restriction the rate of body weight gain in effectively lesioned rats on chow or milk was not significantly different from the rate of weight gain during the restriction (p=.171 U=4, n_1= 4, n_2=4 for milk p=.310, U=21, n_1= 7, n_2=7 chow; Mann Whitney U Test, two-tailed), but was significantly less than the rate of weight gain during restriction in the animals fed the HF diet (p=.016, U=2, n_1=5, n_2=5; Mann Whitney U Test, two tailed). The rate of weight gain of the animals fed milk was significantly less (p=.02, U=4, n_1=4, n_2=8; Mann Whitney U Test, two-tailed) than the rate of weight gain of animals fed the same diet ad libitum in a previous study (Becker & Kissileff, 1974; M=6.5 g/day, R-3.0-9.0).

Nonlesioned rats, with the exception of two groups, returned to their pre-restricted rats of weight gain (p=.50, U=10.5, n_1=4, n_2=4 for former LA rats on milk; p=.50, U=4, n_1=3, n_2=3 for former DA rats on chow; p cannot be calculated, n too small for animals on HF; Mann Whitney U Test, two-tailed). The two groups that were exceptional were the DA animals on milk which had a rate of body weight

gain significantly less than before the restriction ($p=.029$, $U=1$, $n_1=4$, $n_2=4$; Mann Whitney U Test, two-tailed) and the LA animals on chow which gained significantly more ($p=.014$, $U=5$, $n_1=4$, $n_2=4$; Mann Whitney U Test, two tailed) body weight than before restriction.

Second Restriction: Period Preceding

Each effectively-lesioned rat on the two solid diets (chow and HF) was consuming more food than any nonlesioned rat prior to the second restriction. Mean daily food intake of the lesioned rats (DA and LA averaged together) exceeded the mean daily food intake of the non-lesioned rats (DA and LA averaged together) by 73.4 percent for the animals on the HF diet and 35.9 percent for the animals fed chow (cf. Figures 26, 27, and 28, 29). However, food intake in the effectively-lesioned rats was less than during the period following the first restriction by a mean of 38.8 (R=30.1-47.1) percent in the animals fed chow and a mean of 28.8 (R=11.9-49.3) percent in the rats on the HF diet, but exceeded the intake before the first restriction and the lesion by a mean of 33.2 (R=2.4-72.3) percent in the animals fed chow and a mean of 58.5 (R=10.1-86.1) percent in the animals on the HF diet. In other words food intake at this time was intermediate between its peak following the lesion and the pre-lesion level.

There were no significant differences between effectively lesioned DA and effectively lesioned LA rats in either light phase intake ($p=.314$, $U=3.5$, $n_1=4$, $n_2=3$ for chow; $p=.20$, $U=1$, $n_1=3$, $n_2=3$ for HF; Mann Whitney U Test, two-tailed), dark phase intake ($p=.429$, $U=5$, $n_1=4$, $n_2=3$ for chow; $p=.10$, $U=0$, $n_1=3$, $n_2=2$ for HF; Mann Whitney U Test,

Figure 26. Means and ranges of daily intakes of the effectively VMH-lesioned DA rats when obese consuming chow (top) and HF (bottom) diets. The wide bars on the left indicate mean daily intake for the 5 days immediately preceding the restriction. The darkened portions of these bars indicate mean daily dark phase intake and the light portions mean daily light phase intake. The dark bars in the center of the figure indicate mean daily intake when access to food was restricted to the dark phase only. The bars on the right side of the figure indicate mean daily intake for the 5 days following the restriction. Their darkened portions indicate mean daily dark phase intake and their light portions mean daily light phase intake. The vertical lines extending above and below each bar on the figure represent the ranges of daily intakes in each animal for that period.

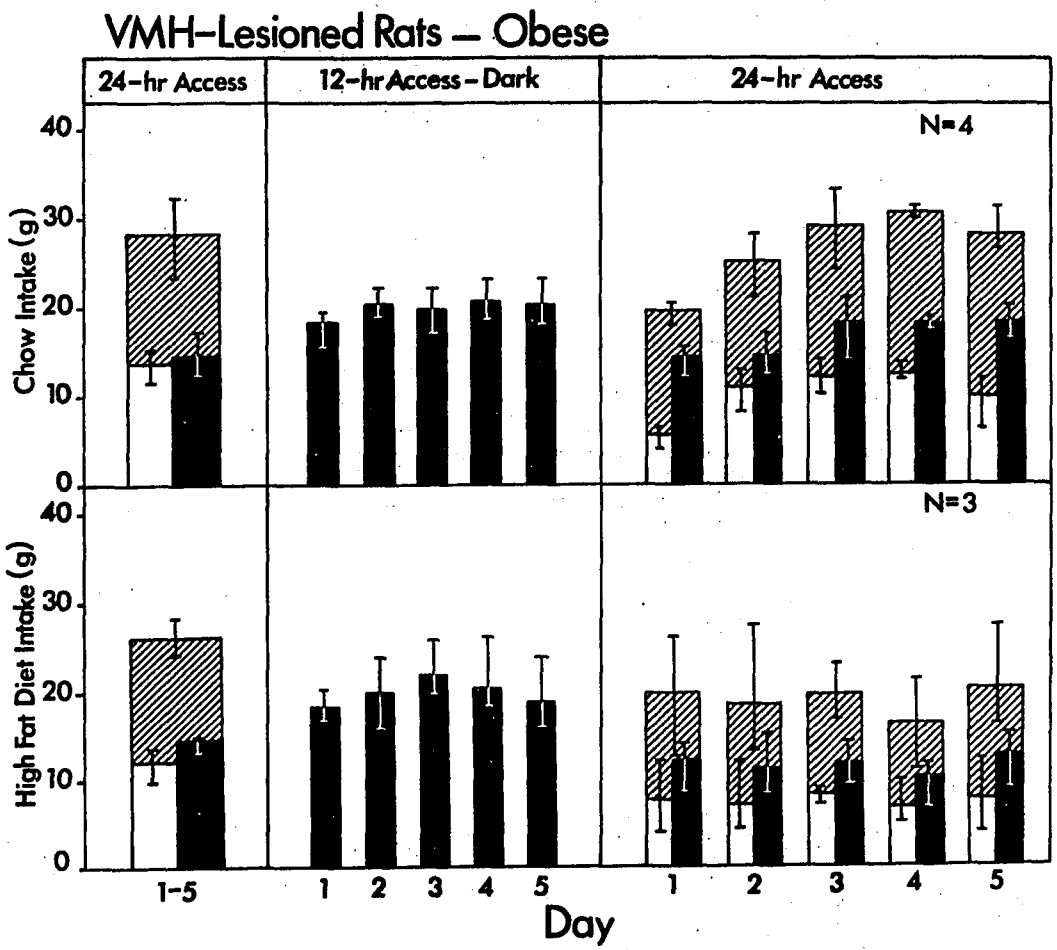

VMH-Lesioned Rats — Obese
24-hr Access
12-hr Access - Dark
24-hr Access
N=4
N=3
Chow Intake (g)
High Fat Diet Intake (g)
0
10
20
30
40
1-5
1
2
3
4
5
Day

Figure 27. Means and ranges of daily intakes of the effectively lesioned LA rats when obese consuming chow (Top) and HF (bottom) diets. The wide bars on the left indicate mean daily intake for the 5 days immediately preceding the restriction. The darkened portions of these bars indicate mean daily dark phase intake and the light portions mean daily light phase intake. The light bars in the center of the figure indicate mean daily intake when access was restricted to the light phase. The bars on the right side of the figure indicate mean daily intake for the 5 days following the restriction. Their darkened portions indicate mean daily dark phase intake and their light portions mean daily light phase intake. The vertical line extending above and below each bar on the figure represent the range of intake in each animal for that period.
/ Data collected on only one rat during the light phase.

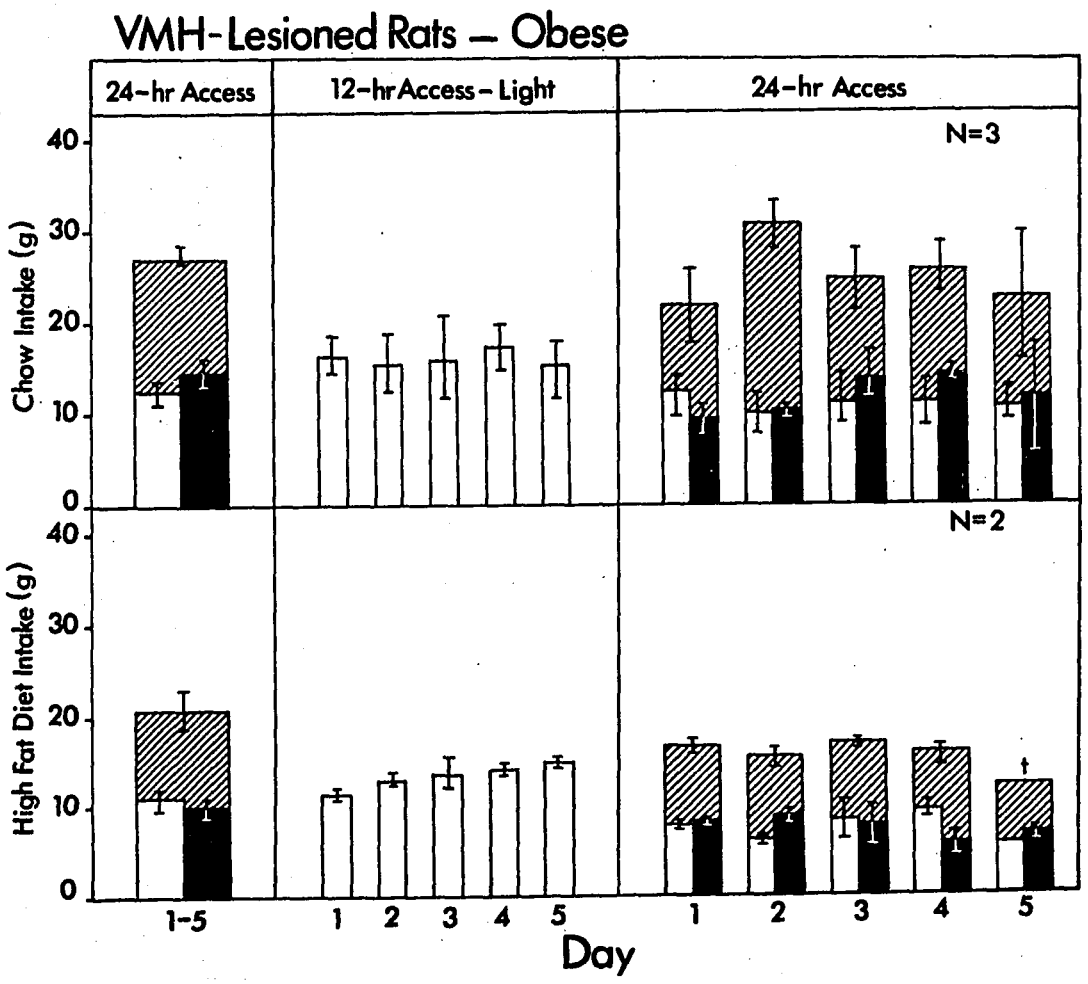

VMH-Lesioned Rats — Obese
24-hr Access
12-hr Access - Light
24-hr Access
N=3
N=2
Chow Intake (g)
High Fat Diet Intake (g)
0
10
20
30
40
1-5
1
2
3
4
5
Day

Figure 28. Means and ranges of intakes of the non-lesioned DA rats before, during, and following the second restriction consuming chow (top) and HF (bottom) diets. For detailed description see Figure 26.

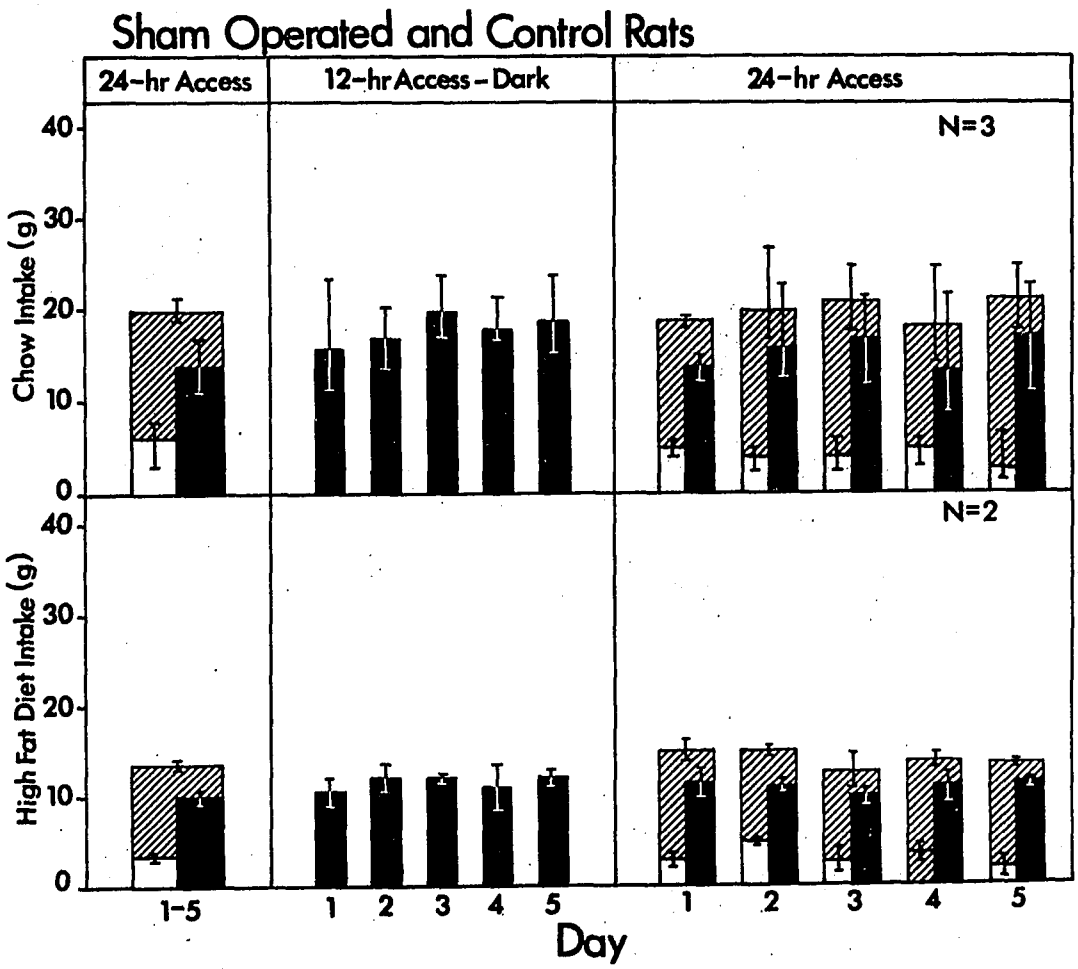
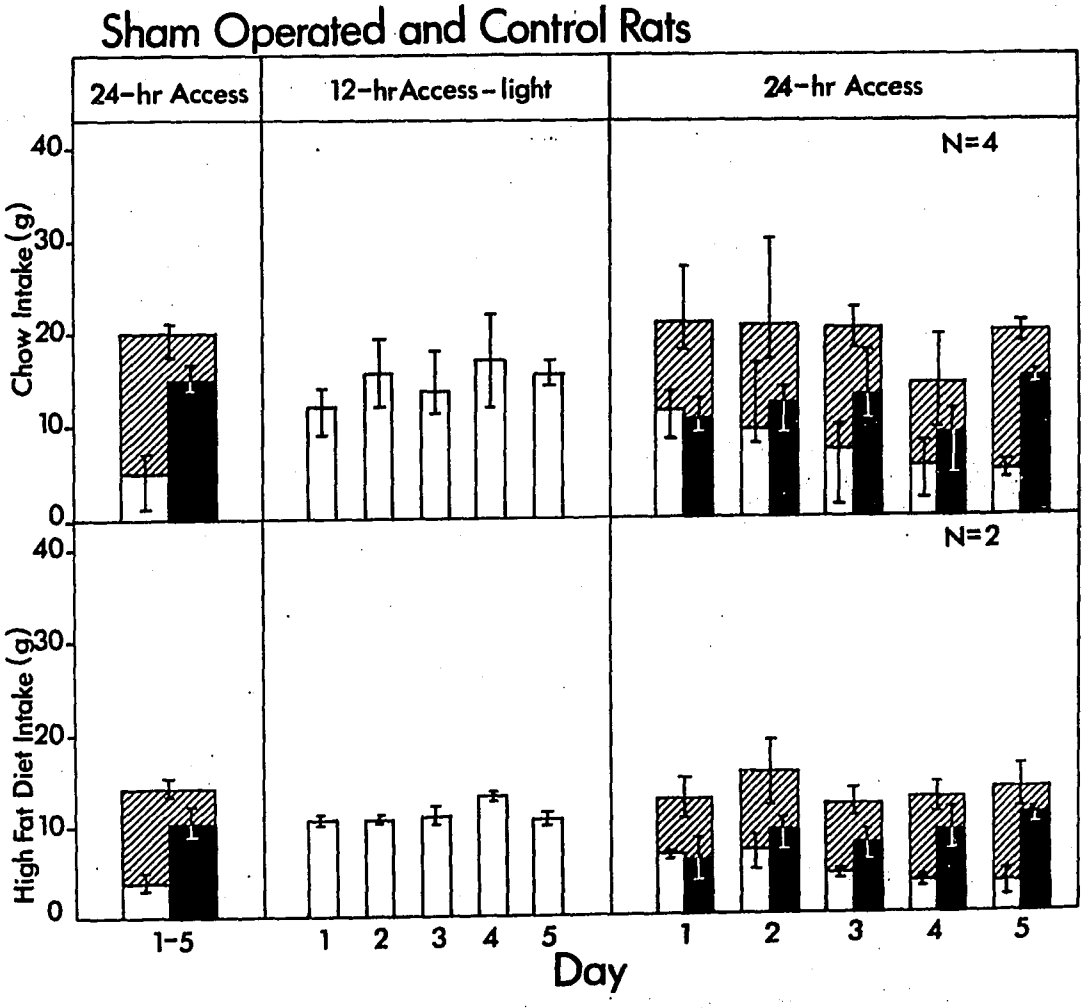

Sham Operated and Control Rats
24-hr Access
12-hr Access - Dark
24-hr Access
N=3
N=2
Chow Intake (g)
High Fat Diet Intake (g)
0
10
20
30
40
1-5
1
2
3
4
5
Day

Figure 29. Means and ranges of intakes of the non-lesioned LA rats before, during, and following the second restriction consuming chow (top) and HF (bottom) diets. For detailed description, see Figure 27.


Sham Operated and Control Rats
24-hr Access
12-hr Access - light
24-hr Access
N=4
N=2
Chow Intake (g)
High Fat Diet Intake (g)
0
10
20
30
40
1-5
1
2
3
4
5
Day

two-tailed) or total intake (p=.314, U=3, n_1=4, n_2=3 for chow; p=.10, U=0, n_1=3, n_2=2 for HF, Mann Whitney U Test, two-tailed) respectively for each of the two diets.

Food intake in all effectively-lesioned animals was divided approximately equally between the light and dark phases (see Figures 26 and 27). In contrast, food intake in the nonlesioned rats was predominantly nocturnal and a mean of 28.1 (R=13.0-40.9) percent of the total daily intake of the animals fed chow and a mean of 26.8 (R=20.1-34.1) percent of the total daily intake of the rats fed the HF diet was consumed while the lights were on. In addition, in nonlesioned rats there were no significant differences between DA and LA animals in light phase intake (p=.20, U=3, n_1=3, n_2=4 for chow; p cannot be calculated, n too small for HF, Mann Whitney U Test, two-tailed), dark phase intake (p=.314, U=4, n_1=3, n_2=4 for chow; p cannot be calculated, n too small for HF, Mann Whitney U Test, two-tailed) or total daily intake (p=.571, U=5.5, n_1=4, n_2=3 for chow; p cannot be calculated, n too small for HF; Mann Whitney U Test, two-tailed) on each of the two diets.

Second Restriction

Effectively Lesioned Rats. Food intake decreased during restriction by a mean of 34.1 (R=15.4-50.0) percent in the rats consuming chow and a mean of 28.3 (R=15.1-38.3) percent in the rats on the HF diet (see Figures 26 and 27). There were no significant differences in the percent reduction in intake between DA and LA animals on both diets (p=.20, U=3, n_1=4, n_2=3 for chow, p=.20, U=1, n_1=3, n_2=2 for HF; Mann

Whitney U Test, two-tailed) although DA animals consumed more food during restriction than LA animals (cf. Figures 26 and 27).

During restriction, however, the mean intake of the effectively-lesioned rats exceeded the mean intake of the nonlesioned rats by a mean of 32.7 percent for the animals fed the HF diet and a mean of 24.1 percent for the animals fed chow (cf. Figures 26, 27, 28, and 29). There was no overlap in the ranges of intakes between lesioned and nonlesioned rats fed the HF diet, but the difference between lesioned and nonlesioned rats fed chow just bordered on being statistically significant ($p=.0641$, $U=11.5$, $n_1=7$, $n_2=7$, Mann Whitney U Test, two-tailed). The critical value of U for significance ($p < .05$) would be 11. Therefore the major effect of the second restriction was to reduce intake more drastically in the effectively-lesioned rats than in nonlesioned rats, and thus reduce the differences between the intakes of the effectively-lesioned rats and nonlesioned rats.

Nonlesioned Rats. The effect of the second restriction in the nonlesioned rats was similar to the first restriction. There were no differences in the percent reduction in food intake which occurred during the first and second restrictions with each diet ($p=.10$, $U=14$, $n_1=7$, $n_2=7$ for chow; $p=.171$, $U=4$, $n_1=4$, $n_2=4$ for HF; Mann Whitney U Test, two tailed; cf. Figures 19, 20, and 28, 29). As in the first restriction, there were no significant differences between the intakes of DA and LA animals with both diets ($p=.314$, $U=4$, $n_1=3$, $n_2=4$ for chow; p cannot be calculated, n too small for HF; Mann Whitney U Test, two-tailed).

Second Restriction: Return to 24-hour Access

Effectively-Lesioned Rats. Upon return to 24-hour access, intakes in the effectively-lesioned rats fed chow returned to approximately the same level as before the restriction (see Table 21). Intake in the animals fed the HF diet did not change from the level during restriction (see Figures 26 and 27) and hence were significantly different from the intake before the restriction (see Table 22). In addition with both diets intake became immediately distributed equally between the light and dark phases in all lesioned rats (see Figures 26 and 27). This equal distribution of intake was accomplished by a reduction in dark phase intake for DA animals and light phase intake for LA animals.

Nonlesioned Rats. Upon return to 24-hour access, food intake returned to the pre-restriction level in all animals. The normal nychthemeral periodicity of food intake returned in all animals (see Figures 28 and 29).

Second Restriction: Body Weight Changes

Before Restriction. Rates of body weight gain in the 5 days before the second restriction ranged from -1.4 to 7.3 g per day in the effectively-lesioned animals. These values were considerably less than the rates of weight gain immediately after the lesion which ranged from 0.60 to 18.8 g per day. With respect to the rate of body weight gain immediately before the lesion, the rates of body weight gain at the second restriction were highly variable (see Table 17). Thus although all the animals were obese at the second restriction (see Table 17), some were in the static phase while others were still gaining weight slowly. However, DA animals but not LA animals gained weight at a significantly greater rate than nonlesioned rats ($p=.114$, $U=2$, $n_1=3$, $n_2=4$ LA animals on chow; p cannot

Table 18. Analysis of Variance Source Table Comparing Intakes During Restriction and Restoration Periods in Effectively-Lesioned Rats Fed Milk During the Dynamic Phase

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	p
Between subjects	1580.25	3			
Within subjects	1737.75	28			
(access conditions) A	480.5	1	480.5	18.78	< .05
Ax subjects within group	76.75	3	25.58		
(days) B	32.50	3	10.83	< 1.0	N.S.*
Bx subjects within group	281.25	9	31.25		
AB	100.00	3	33.33	< 1.0	N.S.*
ABx subjects within group	766.75	9	85.19		
TOTAL	3318.00	31			

*N.S. = not significant

Table 19. Analysis of Variance Source Table for Comparing Intakes During Restriction and Restoration Periods in Effectively-Lesioned Rats Fed Chow During Dynamic Phase

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	p
Between subjects	1939.490	6			
Within subjects	1800.064	35			
(access condition) A	987.945	1	987.945	18.34	< .01
A x subjects within group	323.186	6	53.864		
(days) B	166.328	2	83.16	16.46	< .01
B x subjects within group	60.626	12	5.05		
AB	48.64	2	24.32	1.36	> .25
AB x subjects within groups	213.338	12	17.778		
Total	3739.554	41			

*N.S. = not significant

Table 20. Analysis of Variance Source Table for Comparing Intakes During Restriction and Restoration in Effectively-lesioned Rats Fed HF Diet During the Dynamic Phase

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	p
Between subjects	470.5965	4			
Within Subjects	1176.5025	35			
(access conditions) A	210.681	1	210.681	19.36	<.01
A x subjects within groups	43.531	4	10.882		
(days) B	200.921	3	66.97	2.77	>. 05
B x subjects within groups	290.191	12	24.18		
AB	34.593	3	11.53	<1.00	N.S.*
AB x subjects within groups	396.584	12	33.049		
Total	1,647.099	39			

*N.S. = not significant

Table 21. Analysis of Variance Source Table Comparing Intake before Second Restriction and Intake following Restriction in Obese Effectively-Lesioned Rats Fed Chow

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	p
Between subjects	208.767	6			
Within subjects	815.295	49			
(period) A	90.525	1	90.53	5.68	< .10
A x subjects within group	95.495	6	15.92		
(days) B	91.606	3	30.53	3.07	< .10
B x subjects within group	178.969	18	9.942		
AB	66.404	3	22.135	1.364	N.S.*
AB x subjects within groups	292.295	18	16.239		
Total	1024.062	55			

*N.S. = not significant

Table 22. Analysis of Variance Source Table Comparing Intake Before Second Restriction and Intake Following Second Restriction in Obese Effectively-Lesioned Rats Fed HF Diet

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	p
Between subjects	294.422	4			
Within subjects	489.94	25			
(period) A	283.361	1	283.361	14.27	<.05
A x subjects within groups	79.436	4	19.86		
(days) B	7.601	2	3.80	<1.00	N.S.*
B x subjects within groups	58.276	8	7.29		
AB	2.245	2	1.13	<1.00	N.S.*
AB x subjects within groups	59.021	8	7.38		
Total	784.36	29			

*N.S. = not significant

Figure 30. Means and ranges of body weight changes of the effectively VMH-lesioned DA rats on two experimental diets before, during, and following the second restriction. The mean rate of body weight change for the five days immediately preceding the restriction is shown on the left-hand side of the figure. Mean rate of body weight change during the restriction is shown in the center of the figure. Mean rate of body weight change following restriction is shown on the right-hand side of the figure. The line above and below each bar represent the range of mean body weight change in individual animals for that period. For complete description of how the rate of body weight gain was calculated, see data analysis section.

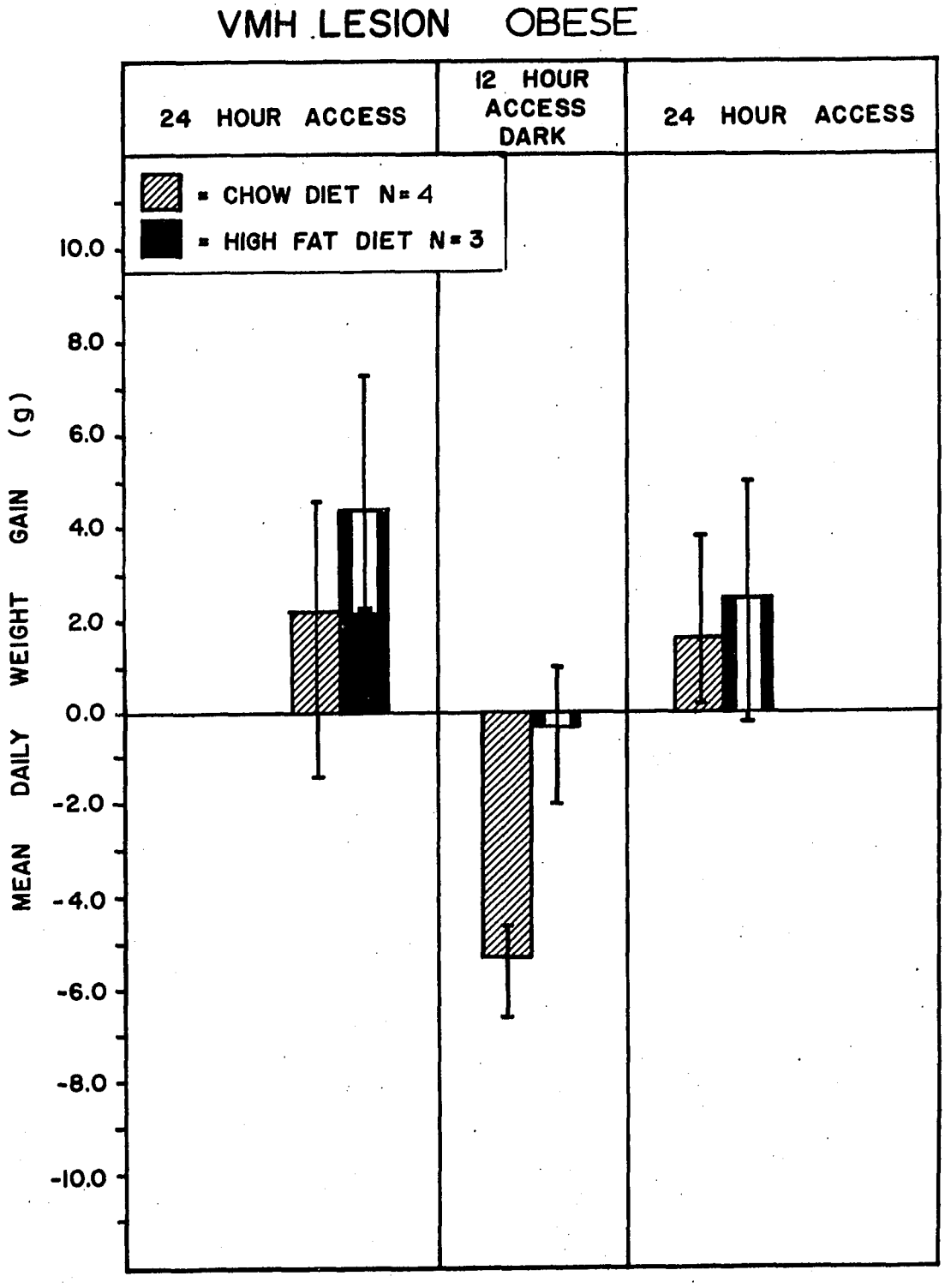
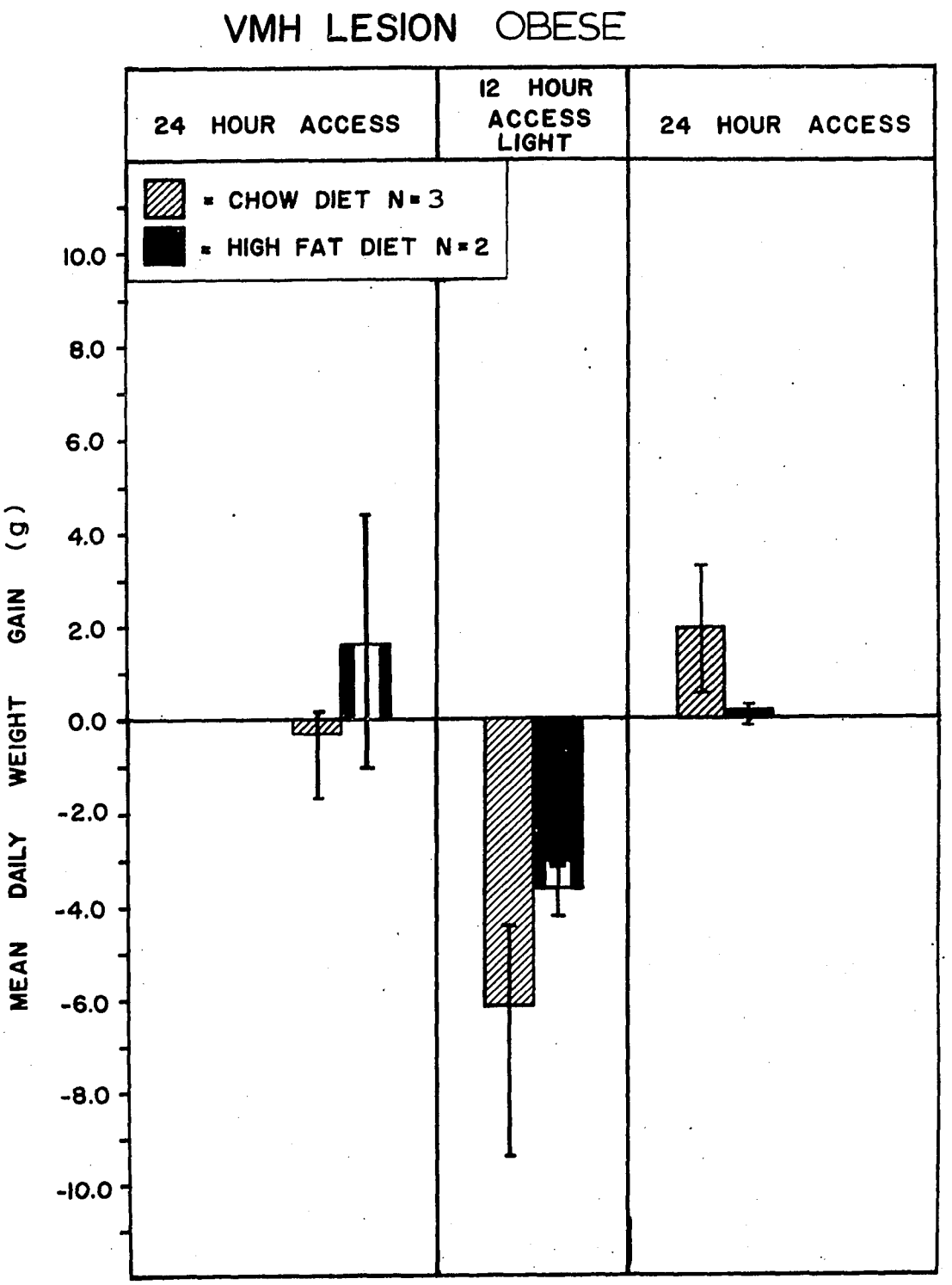

VMH LESION OBESE
24 HOUR ACCESS
12 HOUR ACCESS DARK
24 HOUR ACCESS
= CHOW DIET N=4
= HIGH FAT DIET N=3
MEAN DAILY WEIGHT GAIN (g)
10.0
8.0
6.0
4.0
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0

Figure 31. Means and ranges of body weight changes of the effectively VMH-lesioned LA rats on two experimental diets before, during, and following the second restriction. The mean rate of body weight changes for the 5 days immediately preceding the restriction is shown on the left-hand side of the figure. Mean rate of body weight change during the restriction is shown in the center of the figure. Mean rate of body weight change following restriction is shown on the right-hand side of the figure. The lines above and below each bar represent the range of body weight changes in individual animals for that period. For complete description of how the rate of body weight gain was calculated, see data analysis section.


VMH LESION OBESE
24 HOUR ACCESS
12 HOUR ACCESS LIGHT
24 HOUR ACCESS
= CHOW DIET N=3
= HIGH FAT DIET N=2
MEAN DAILY WEIGHT GAIN (g)
10.0
8.0
6.0
4.0
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0

be calculated, n too small for LA animals on high fat; no overlap between the rate of weight gain of DA lesioned and nonlesioned animals; Mann Whitney U Test, two-tailed.

During Restriction. During restriction all effectively lesioned rats on both diets either lost body weight or their rate of body weight gain was severely depressed (see Figures 30 and 31). There was no overlap in the rate of body weight gains before and during the restriction in effectively lesioned rats. All effectively-lesioned animals fed chow lost more weight than all nonlesioned rats fed chow. The rate of weight change in the animals fed the HF diet was not significantly different between effectively lesioned and nonlesioned rats ($p=.402$, $U=8.5$, $n_1=5$, $n_2=4$, Mann Whitney Test, two-tailed).

There were no differences between access conditions in either effectively-lesioned and nonlesioned rats and thus animals deprived during the dark and animals deprived during the light lost approximately the same amount of body weight during the restriction (see Figures 30 and 31).

Following Restriction. The rates of body weight gain in the effectively-lesioned animals in the 5 days following the restriction were similar to rates of gain before the restriction and ranged from -0.2 to 5.0 g per day (see Figures 30 and 31). The rate of body weight gain in the nonlesioned rats returned to approximately the same level as before the restriction.

Histology

Obese rat LC 2 had its lesion centered in the anterior portion of the ventromedial hypothalamus approximately at the level of plate #4890 (Konig & Klippel, 1943). The lesion destroyed almost the entire anterior portion of the ventromedial nucleus, the dorsomedial nucleus, and damaged the third ventricle. The lesion extended anteriorally to the level of the arcuate nucleus approximately at the level of plate #5150 (Konig & Klippel, 1963) and posteriorally to the level of the central part of the ventromedial nucleus approximately to the level of plate #4230 (Konig & Klippel, 1963). Obese rat LC 4 had a lesion centered in the middle portion of the ventromedial nucleus (Konig & Klippel, 1963, plate #4620). This lesion destroyed most of the ventromedial nucleus, the dorsomedial nucleus, and extended dorsally to the zona inserta. This lesion extended anteriorally to the level of the anterior ventromedial nucleus (Konig & Klippel, 1963, plate #4890) and posterially to the level of the central part of the ventromedial nucleus (Konig & Klippel, 1963), plate #4890). Obese rat LC 13 had a large lesion centered in the ventromedial nucleus at the level of plate #4380 Konig & Klippel, 1963). This lesion destroyed all of the ventromedial nucleus and dorsomedial nucleus. The lesion extended anteriorally to the level of the arcuate nucleus (Konig & Klippel, 1963, plate #5150) and posterially to the level of the beginning of the anterior pole of the subthalamic nucleus (Konig & Klippel, 1963, plate #3990).

Moderately obese rat, LC 3, had a large lesion centered at the level of the ventromedial nucleus (Konig & Klippel, 1963, plate #4110).

At this level the destroyed bilaterally almost all of the ventromedial nucleus and dorsomedial nucleus. Moderately obese rat LC 1 had a small lesion centered in the ventromedial nucleus (Konig & Klippel, 1963, plate #4380).

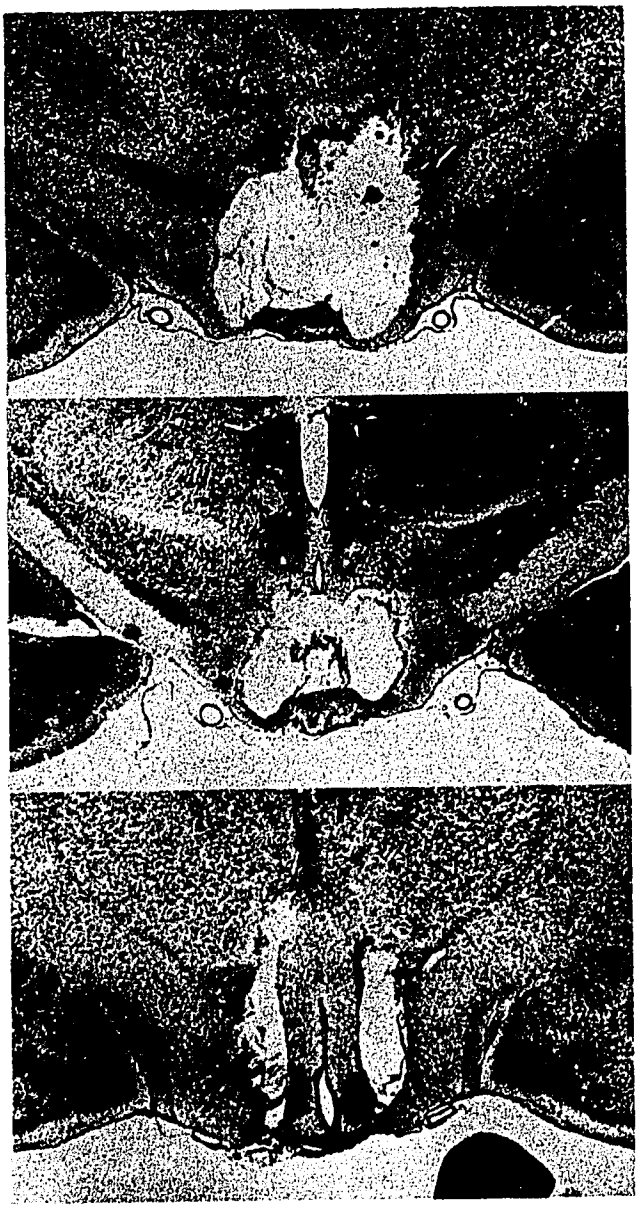
Obese rat, LC 14, had a large lesion centered at the level of the ventromedial nucleus (Konig & Klippel, 1964, plate #4380). The lesion destroyed all of the ventromedial nucleus as well as the dorsomedial nucleus. The lesion extended anteriorally to the anterior portion of the ventromedial nucleus (Konig & Klippel, 1963), plate #4890) and posterially to the level of the beginning of the anterior pole of the subthalamic nucleus (Konig & Klippel, 1963, plate #3990). LC 20 had a large lesion centered at the level of the anterior portion of the ventromedial nucleus (Konig & Klippel, 1963, plate #4620). The lesion spared the ventral portion of the ventromedial nucleus,but completely damaged the dorsomedial nucleus. The lesion extended anteriorally to the level of the arcuate nucleus (Konig & Klippel, 1963, plate #5150) and posteriorally to the level of the beginning of the anterior pole of hte subthalamic nucleus. LC 26 had a large lesion centered at the level of the anterior portion of the ventromedial nucleus (Konig & Klippel, plate #4810). At this level the lesion destroyed almost all of the anterior ventromedial nucleus, and portions of the dorsomedial nucleus. The lesion extended posteriorally to the central part of the ventromedial nucleus and anteriorally to the subthalamic nucleus. LC 28 and LC 32 had large lesions centered at the level of the ventromedial nucleus (Konig & Klippel, 1963, plate #4380). The lesion in LC 28 appeared to spare a small portion of the ventromedial nucleus, but destroyed all of the dorsomedial nucleus. The lesion in LC 32 destroyed all of the ventromedial nucleus at this level. The lesion in LC 28 extended anteriorally from the level of the anterior portion of the ventromedial

nucleus (Konig & Klippel, 1963, plate #4890) and posteriorally to approximately the anterior pole of the subthalamic nucleus. The lesion in LC 32 had the same posterior extent, but extended further anteriorally into the anterior hypothalamus.

Moderate obese rat, LC 18, had partial damage in the ventromedial nucleus. Its lesion was centered extremely posterior of the ventromedial nucleus centered at the level of the mammallary bodies. Moderate obese rat, LC 24, had damage in the posterior portion of the ventromedial nucleus, but the lesion was assymmetrical and appeared to spare most of the ventromedial nucleus on one side.

LC 34, 38, and 40 all had large lesions centered at the level of the ventromedial nucleus (Konig & Klippel, 1963, plate #4380) which destroyed all or most of the ventromedial nucleus and the dorsomedial nucleus. These lesions extended anteriorally to the anterior portion of the ventromedial nucleus and posterially to the anterior pole of the subthalamic nucleus. LC 36 and LC 44 had large lesions centered further posterior in the ventromedial nucleus (Konig & Klippel, 1963, plate #4110). These lesions destroyed all or most of the ventromedial nucleus, the dorsomedial nucleus and portions of the third ventricle. The lesions extended anteriorally to the center part of the ventromedial nucleus and posteriorally to the anterior pole of the subthalamic nucleus.

Figure 32. Photomicrographs of coronal sections at the point of maximal extent of the lesion of obese rats LC 36 (top), LC 28 (middle), and LC 4 (bottom). These animals were fed HF, chow and milk diets respectively.



Discussion

There were two significant results of this study. First, VMH-lesioned rats in the five days immediately following the lesion overate and gained excessive amounts of body weight despite restriction to feeding only during the dark phase. Second, when the VMH-lesioned animals became obese, they were much less responsive to the effects of the restriction. The first result is not surprising because in several previous studies (Balagura & Devenport, 1970; Becker & Kissileff, 1974; Brooks et al., 1946) it has been shown that the hyperphagia following VMH lesions is not entirely due to increased light phase feeding. In each of these studies, at least part of the hyperphagia could be attributed to overeating during the dark phase. However, the amount of overeating that occurred during the dark contributed only a small amount to the total amount consumed, and most of the excess intake was eaten during the light phase period. The major point of this experiment is that VMH-lesioned rats not only overeat while limited to feeding during the dark but that they can maintain a high degree of hyperphagia and excessive rate of body weight gain and overeat by an equal amount when restricted to feeding only during the light phase. Furthermore, the effect of restriction to 12 hours of food access is the same in both dynamic VMH-lesioned and normal rats and food intake is reduced by the same percentage in both.

There are two alternative interpretations of these results. First, it may be that hyperphagia following VMH lesions cannot be

explained as a primary disturbance in the nychthemeral cycle of lipolysis and lipogenesis as proposed by LeMagnen et al. (1973). In this case one would have to look to other mechanisms for the cause of the hyperphagia. A second interpretation is that the extreme procedure of preventing the rats from eating in the light, when the lipogenic phase had replaced the lypolytic phase introduced a new variable, namely deprivation, to which the animals responded with exaggerated food intake when food was restored during the dark. In other words a positive result (failure of hyperphagia) during restriction, could have been taken as evidence that the lipolysis-lipogenesis cycle was directly involved in the producing of the hyperphagia, but the present negative result cannot refute the possibility that disruption of the cycle is the cause of the hyperphagia when the animals are eating ad libitum. The second interpretation introduces a more complicated explanation because of its implicit assumption that the mechanism which controls intake during food restriction is different from the one operating during ad libitum access. Until sufficient data supporting the hypothesis that separate mechanisms exist controlling food intake under various conditions of deprivation, we must accept the first interpretation because of its simplicity.

Although we have shown here that the loss of nychthemeral periodicity of feeding is not essential to hyperphagia and in the previous experiment that hyperphagia does not always result in disruption of nychthemeral periodicity, the coincidence of hyperphagia and the loss of nychthemeral periodicity of feeding in the

VMH-lesioned rat still needs to be explained. One possible explanation is that the two phenomena are due to damage in separate and independent neural systems. This idea is supported by the recent work of Bernardis (1973) in which a disruption in the day-night rhythm of feeding was produced without hyperphagia in weanling rats with discrete lesion in the dorsomedial nucleus. The dorsomedial nucleus lies adjacent and just dorsal to the ventromedial nucleus, and lesions which produce hyperphagia probably also damage the cells or connections to the cells involved in controlling the nychthemeral periodicity of feeding. The disruption in the nychthemeral periodicity of feeding may be a primary effect of damage to these cells and the increased feeding during the light phase period could cause an increased respiratory quotient and a decreased plasma concentration of free fatty acids during the light phase. Both the increased respiratory quotient and decreased plasma concentration of free fatty acids during the light phase have been accepted as indications of the disturbances in the daily cycle of lipogenesis and lipolysis in VMH-lesioned rats by Le Magnen, et al. (1973). Unfortunately from LeMagnen et al., (1973) we are unable to determine whether these are primary effects of the lesion or secondary to the increased feeding during the light phase.

The possibility that the disruption in the nychthemeral periodicity of feeding and the hyperphagia are due to damage in separate and independent neural systems is not surprising because

the hypothalamus appears to operate independently of homeostatic functions in generation and entrainment of other behavioral rhythms. Stephan and Zucker (1972) have recently shown that lesions in the suprachiasmatic nuclei of the hypothalamus permanently disrupt the nychthemeral periodicity of drinking without affecting the total amount of drinking.

Another possible reason why VMH-lesioned animals distribute their food intake evenly throughout the 24 hours despite the ability to consume most of it within 12 hours is because this may be the easiest means of expressing the hyperphagia. It is possible that when food intake is increased, there exists an order of priorities of alterations in the meal pattern. If ease of expression were the basis for this order of priorities and increasing intake during the light phase were the easiest means of expression for the hyperphagia, it would be expected that all hyperphagia rats would exhibit this alteration before other alterations. However, in lactating rats the opposite occurs. These animals enlarge their meal sizes, shorten the periods of satiety following meals of a given size, and do not exhibit a disruption in the normal nychthemeral periodicity of feeding until food intake has more than doubled. At this time the disruption is only slight and occurs only in the animals fed chow and not in the animals fed milk. These findings also would refute the possibility that VMH-lesioned rats eat more during the light phase because they are unable to increase their dark phase intake sufficiently.

The second important finding in this experiment was that the response of the VMH-lesioned rats after being allowed to gain weight and become obese was dramatically different from their response initially following the lesion. When obese, the lesioned animals reduced their intakes and rate of body weight gain. The response to the restriction in the obese lesioned animals was not the same as the response of the non-lesioned rats. Although both obese and nonlesioned rats had decreased food intakes and reduced rates of body weight gain during the restriction, the percentage decreases in intake in the obese lesioned animals was greater than in the non-lesioned rats. Thus the obese lesioned animals were not responding as "normal" rats whose body weight were being regulated at an elevated level. It should be noted also that the common difference between animals in the first and second restriction conditions is the elevated level of body weight at the second restriction and not in the rate of weight gain which was highly variable at the second restriction. This is a most important point because it provides a major challenge to the idea that VMH-lesioned rats overeat because "VMH damage . . . elevat[es]theupper body weight appetite setpoint" (Sclafani & Kluge, 1974, p.42). If set point alteration were the dysfunction driving food intake up, then the response of the obese animals to 12 h of food access ought to have been the same as the normals.

This result would suggest that the disturbance in the nychthemeral periodicity of feeding is more critical to the maintenance of food intake in obese VMH-lesioned rats than dynamic VMH-lesioned rats.

However, the obese lesioned animals did consume more food than the nonlesioned rats both before and during the second restriction. Since the second restriction did not completely abolish the hyperphagia, we can assume that the obese lesioned animals possess at least some capacity to respond to restriction. Thus these results do not support a recently proposed neural model of feeding behavior (Panksapp, 1971).

Obese lesioned animals, however, apparently are unable to respond as well as dynamic hyperphagic lesioned animals to the challenge of limited access to food, and therefore must not be able to alter other meal parameters sufficiently. Thus the obese lesioned rats may lose some of the ability to increase either meal size or shorten the periods of satiety following meals of a given size in response to the challenge of limited access.

The inability to respond to the limited access could be the result of the obesity. It has been well established in VMH-lesioned rats (Marks & Remley, 1972; Porter & Allen, 1972; Scalfani & Kluge, 1974) and neurologically normal rats (Quartermain & Kissileff, unpublished data) that the ability to respond on food motivated tasks is affected by obesity. In addition the changes in the meal pattern in VMH-lesioned rats as they become obese suggest that the ability to alter meal size might be reduced as the animals become obese. As VMH-lesioned rats become obese, meal sizes decrease and the periods of satiety following meals of a given size (satiety ratios) increase (Becker & Kissileff, 1974). The changes

in the ad libitum meal pattern may bear no relationship to the animal's ability to alter its meal pattern in response to limited access. However, the changes in the ad libitum meal pattern which possibly result from the obesity are the opposite of the changes the animal must make to compensate for the limited access. Thus it seems conceivable that the animal's inability to respond to limited access could be the result of the obesity's effect on the meal pattern. Furthermore, normal rats respond to obesity by decreasing meal size and normal rats made obese by force-feeding do not respond to food deprivation by increasing meal size (Kissileff & Quartermain, 1973) as rats of normal body weight do (Levitsky & Collier, 1969).

The failure of VMH-lesioned animals to maintain intake during the restriction may not be due to the obesity, but rather the result of a form of neural recovery or reorganization which occurs following the lesion. It has recently been shown that VMH-lesioned rats reduced to their presumed normal body weights by food limitation, when allowed to re-feed with ad libitum access do not exhibit the enlarged meals which occur following the lesion (Becker & Kissileff, 1974b).

In summary, the results of this experiment show that the extreme hyperphagia and excessive body weight gains following VMH lesions are not affected by restriction to 12 h of access to food. Thus the disturbance in the daily cycle of lipogenesis and lipolysis

as proposed by LeMagnen et al (1973) cannot be the only cause of the hyperphagia following a VMH lesion. Obese VMH-lesioned rats, however, are not able to maintain intake during restriction to 12 h of food access, but still consume more food than nonlesioned rats. This suggests the possibilities that the ability of the VMH-lesioned rat to respond to the challenge of restricted access to food is reduced by obesity or a form of neural recovery or reorganization following the lesion (Becker & Kissileff, 1974b).

General Discussion and Summary

From previous investigations of meal patterns in rats at least four possible explanations for hyperphagia have merged. Briefly these are as follows:

1) Decreased Post-prandial Satiety. Hyperphagia results from a defect in the mechanism controlling meal termination (satiety) without damage to the mechanism controlling meal initiation (hunger; Miller et al., 1950). The result is overeating caused by enlarged meals without a compensatory decrease in meal frequency (Brooks et al., 1946; Teitelbaum & Campbell, 1958).

2) Decreased Intermeal Suppression of Feeding. The animal becomes less sensitive to the normal signs which maintain satiety between meals, or, alternatively, there is a more rapid decay of satiety. The hyperphagia results from the animal feeding sooner than would normally be expected after meals of a given size (Thomas & Mayer, 1968).

3) Resetting Body Weight Set Point. The animal becomes less sensitive or less responsive to a signal correlated with obesity which normally acts to inhibit food intake (Hoebel & Teitelbaum, 1966). Thus, "a higher weight level is required to inhibit food intake, and the animal overeats until it reaches that level" (Hoebel & Teitelbaum, 1966, p. 193). A more recent explanation for the hyperphagia syndrome is that "VMH damage elevate[s] the upper body weight appetite set point . . .[and]the VMH animal overeats palatable food because its appetite is disinhibited and stops overeating in the static phase because its obese weight activates

the appetite inhibitory set point mechanism" (Sclafani & Kluge, 1974, p. 42).

4) Increased Light Phase Intake. Increased intake results from a disturbance in the daily cycle of lipolysis and lipogenesis (LeMagnen et al., 1973). This causes a disruption in the normal nychthemeral periodicity of feeding (LeMagnen & Devos, 1970). Thus animals, "are in fact, hyperphagic because they eat in daytime the same amount as in the night" (LeMagnen, 1971, p. 245). In other words, the overeating is primarily the result of an increase in feeding during the light phase without a compensatory decrease in feeding during the dark phase (Brooks et al., 1946; Balagura & Devenport, 1970; Becker & Kissileff, 1974; Kakolewski et al., 1971).

In the present investigation the fourth explanation, increased light phase intake without compensatory reduction in dark phase intake, was eliminated as the sole causal reason for the increased intake. This was shown in two ways. First, in the third experiment it was demonstrated that lactating rats approximately double intake, but do not have a disruption in the nychthemeral periodicity of feeding. This demonstrates that the disruption in day-night feeding is not essential to hyperphagia. Second, it was shown in experiment 4 that in the five days immediately following a VMH lesion that overeating and excessive body weight gain could still occur despite restriction to only 12 hours of access to food.

In addition, the data from these studies do not support the idea that "VMH damage elevate[s] the upper body weight appetite set point" (Sclafani & Kluge, 1974, p. 42), and suggest instead that the alterations

in food and body weight are due to disturbances in either the feedback from detectors for body weight, body lipid content, short-term satiety, or to the systems which respond to these signals. If food intake and meal patterns are controlled by a simple control systems model in which intake is adjusted to a difference (error signal) between an actual value of body weight or some function of it and a set value of body weight or some function of it (i.e. body weight set point of any type, upper, lower, etc.), then when the animal is at the set point, no error signal will be produced and food intake and mealtaking will be the same regardless of actual body weight. If body weight set point is elevated, then alterations in the meal pattern should occur until the animal reached that set point and when the animal reached that set point, all of the alterations would return to normal much in the same way that the meal pattern of normal rats is altered following weight loss due to deprivation (Levitsky, 1970; Levitsky & Collier, 1968) or body weight elevation resulting from force-feeding (Kissileff & Quartermain, 1973). However, there is no evidence that when the animal reaches its elevated body weight and is "regulating" around its new set point, that the alterations in the meal pattern accompanying the hyperphagia disappear and the animal's feeding behavior becomes normal as seen in two experiments. First, in experiment 1 it was shown that when VMH-lesioned rats became obese, the animal which ate the largest meals also had the largest intake. This correlation was not present before the lesion. Second, in experiment 4 it was shown that there was a greater percentage decrease in food intake during restriction to 12 hours of access in obese VMH-lesioned rats than in normal rats. These results show that obese VMH-lesioned rats are not merely normal rats whose body weight is being regulated at an elevated level and suggest that hyperphagia is not merely the result of a resetting body weight

set point. Rather it would appear that the controls of eating are uncoupled from their metabolic consequences.

Failing to account for hyperphagia with the last two suggested explanations, can the other two suggested explanations adequately account for the overeating? Let us therefore examine the possibilities that increased intake results from either decreased post-prandial satiety or decreased intermeal suppression of feeding. The decrease in post-prandial satiety suggests that the predominant change accompanying increased intake is a lessened sensitivity to the inhibitory signals which normally act to terminate ongoing feeding. Evidence would suggest that these signals derive from two separate sources; the gut and the body fat.

Evidence which has implicated the gut and particularly the duodenum in producing inhibitory signals which terminate ongoing feeding comes from experiments which have shown that feeding is inhibited following the infusion of either nonnutritive bulk or hypertonic solution into the duodenum (Ehman, Albert, & Jamieson, 1974). More recent studies (Gibbs, Young, & Smith, 1973; Smith, Gibbs, Young, 1974) have suggested that these inhibitory signals might result from the release from the duodenal mucosa of the gut hormone, cholecystokinin (CCK), because injections of CCK have been effective in inhibiting food intake by decreasing the size of meals following deprivation with both solid and liquid diets (Gibbs, et al., 1973) and by terminating feeding in rats with open gastric fistulae who do not ordinarily show satiety (Smith, et al., 1974). However, little else about the operation of this hormone is known and the mechanism for satiety signals which originate in the gut.

Even less is known about the participation of the body fat in the termination of meals. However, the following evidence suggests the

existence of a mechanism which affects intake by relating the termination of feeding to the animal's fat stores and maybe even its energy needs. When the body weight of an animal is reduced by fasting, the increased intake during the refeeding, is primarily caused by increase in meal size (Levitsky, 1970; Levitsky & Collier, 1968). When the body weight of an animal is increased by force-feeding, the decreased intake which results following the force-feeding is accomplished by a decrease in meal size (Kissileff & Quartermain, 1973).

A decreased sensitivity to the inhibitory signals which ordinarily act to terminate ongoing feeding could not by itself by the sole causal mechanism for increased intake. Several investigators (Balagura & Coscina, 1968; LeMagnen & Tallon, 1966; Snowden, 1969; Thomas & Mayer, 1968) have shown a positive significant correlation between meal size and the intervals which follow it. Enlarged meals which result from deficits in the mechanisms which normally terminate ongoing feeding should produce increases in the length of following intervals, and hence the animal should maintain intake by eating fewer meals. Therefore, for total intake to increase either one of the following must occur. Either the decrease in post-prandial satiety must be accompanied by a decrease in the intermeal suppression of feeding or the relationship between meal size and the interval following must disappear which indicates an uncoupling of the mechanism which normally relates meal size to the interval which follows it. It has been shown that there is a positive significant correlation in hyperphagic VMH-lesioned animals (Thomas & Mayer, 1968). This finding suggests that there are two alterations in the controls causing the hyperphagia: 1) decreased post-prandial satiety; and

2) decreased intermeal suppression of feeding. However, when VMH-lesioned rats are fed less than maximally palatable diets, meals are reduced to the same sizes as they are in normal rats (Sclafani, 1974). This finding could be interpreted as evidence that VMH-lesioned rats only increase meal size in response to an elevation of appetite setpoint which somehow makes normal food more palatable. Since in the normal rat increased palatability leads to increased meal size, a cognitive palatibility increase, not a satiety failure is the cause of the large meals. However, this conclusion was based upon interpretation of the data using a 10-minute criterion to define intermeal interval. The use of a longer criterion might have produced different results and a different conclusion. As shown in experiment 1 the small meals in the VMH-lesioned rat could have been the result of many short interruptions between feeding bouts because when barriers to ingestion are posed, animals are prone to interrupt feeding more frequently (Kissileff, 1970).

The two alterations which apparently are responsible for the hyperphagia are the result of either a decreased sensitivity to the normal signals which act to terminate feeding and keep feeding suppressed between meals or an inability to properly interpret these signals. It has recently been speculated that these two mechanisms can be accounted for by two kinds of cells in the VMH; those which terminate ongoing feeding (T cells) and those which keep feeding suppressed betwen meals (S cell; Becker & Kissileff, 1974).

One of the major findings of this study was that the expression of increased intake can differ qualitatively and there exists no single constellation of alterations in meal pattern accompanying increased intake. These results suggest that either there is no systematic relationship between increases in food intake and changes in meal parameters or a conclusion opposite to the reasoning above; specifically that the mechanisms for each of the instances of increased intake are different. The implication of this conclusion is that there might be other inhibitory systems for feeding in the brain in addition to the system located in the ventromedial hypothalamic area or along the noradrenergic pathway (Ahlskog & Hoebel, 1973; Kapatos & Gold, 1973). This would be consistent with research which has shown that structures other than the VMH, specifically the septum (Donovick, Burright & Helson, 1969; Singh & Meyer, 1968) and hippocampus (Ehrlich, 1963), appear to exert some inhibitory influence upon food intake in rats. However, the destruction of these structures and the presumed loss of their inhibitory influence does not produce an increase in intake comparable to that following damage to the ventromedial hypothalamic area. Thus evidence that inhibitory systems for feeding outside of the ventromedial hypothalamic area are involved entirely in producing the increased intake seen in this investigation appear slim. However, it is possible that some of these structures participate in producing the increased intake and alterations in meal parameters in addition to the ventromedial hypothalamic area. In order to definitively determine whether this might have occurred further research should be directed toward examining

the changes in meal patterns produced by the loss of inhibitory influences on feeding from extra-hypothalamic structures.

While the entire constellation of alterations in meal pattern accompanying increased intake in VMH-lesioned, genetically obese, and lactating rats were not the same, there were, however, two alterations that were present in the meal patterns of each. These were: 1) increased meal size; and 2) shortened period of satiety following meals of a given size. In a previous study (Becker & Kissileff, 1974) the second deficit in feeding behavior was expressed as a decrease in the mean daily satiety ratio (interval to the following meal divided by the size of the present meal). While it would appear that this statistic might be a useful descriptive index for this deficit, a re-evaluation of its usefulness has shown that it is merely another means of expressing total intake consumed over a specified period of time. Thus any time intake is increased the satiety ratio decreases. The usefulness of the satiety ratio appears limited to evaluating a single meal or feeding behavior over a very short period of time.

The existence of two common alterations in meal pattern would suggest the possibility of two common deficits responsible for the increased intake: 1) decreased post prandial satiety; and 2) decreased intermeal suppression of feeding.

In summary, the data from this investigation do not support the idea that hyperphagia results from either increased light phase intake or a resetting of the body weight set point. Rather it appears that the causal mechanisms for hyperphagia are : 1) decreased post-prandial

satiety; and 2) decreased intermeal suppression of feeding. The appearance of enlarged meals and shortened period of satiety following meals of a given size (satiety ratios) in VMH-lesioned, genetically obese, and lactating rats indicates that these two expressions may be common to several forms of increased intake. The physiological mechanism for hyperphagia has yet to be determined. However, the present investigation has suggested where and when the putative inhibitory signals may arise. It remains future work to determine the origin and exact nature of the inhibitory signals that suppression food intake, their pathways to the brain, and the specific cells these signals impinge upon.

APPENDIX A

Extension of a two-factor Analysis of Variance with Repeated Measures on Each Factor[1]

[1]This statistical test is a modification of the test presented in Winer (1971). The modification was the work of Professor Eugene Michels of the Department of Physical Therapy, University of Pennsylvania

Extension of a two-factor ANOVA with Repeated Measures on Each Factor

Subjects	Conditions A_1				A_2			
	Days: B_1	B_2	B_3	B_4	B_1	B_2	B_3	B_4
1								
2								
3								
4								

I. Computational Formulas and Partitioning the Degrees of Freedom:

Source	Sum of Squares	Degrees of Freedom	Mean Square*	F
Total	$\Sigma\Sigma\Sigma(x)^2 - \frac{(\Sigma\Sigma\Sigma x)^2}{abn}$	abn-1		
Between S_s	$\frac{\Sigma(\Sigma\Sigma x)^2}{ab} - \frac{(\Sigma\Sigma\Sigma x)^2}{abn}$	n-1		Not Testable
Within S_s		n(ab-1)		
A	$\frac{\Sigma(\Sigma\Sigma x)^2}{bn} - \frac{(\Sigma\Sigma\Sigma x)^2}{abn}$	a-1		
AxS_s	$\frac{\Sigma\Sigma(\Sigma x)^2}{b} - \frac{\Sigma(\Sigma\Sigma x)^2}{ab} - \frac{\Sigma(\Sigma\Sigma x)^2}{bn} + \frac{(\Sigma\Sigma\Sigma x)^2}{abn}$	(a-1)(n-1)		$\frac{MsA}{MsA \times S_s}$
B	$\frac{\Sigma(\Sigma\Sigma x)^2}{an} - \frac{(\Sigma\Sigma\Sigma x)^2}{abn}$	b-1		$\frac{MsB}{MsB \times S_s}$
BxS_s	$\frac{\Sigma\Sigma(\Sigma x)^2}{a} - \frac{\Sigma(\Sigma\Sigma x)^2}{ab} - \frac{\Sigma(\Sigma\Sigma x)^2}{an} + \frac{(\Sigma\Sigma\Sigma x)^2}{abn}$	(b-1)(n-1)		

*Mean Square = Sum of Squares/Degrees of Freedom

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
AB	$\frac{ab\ n}{\Sigma\Sigma(\Sigma x)^2}{n} - \frac{b\ an}{\Sigma(\Sigma\Sigma x)^2}{an} - \frac{a\ bn}{\Sigma(\Sigma\Sigma x)^2}{bn} + \frac{abn}{(\Sigma\Sigma\Sigma x)^2}{abn}$	(a-1)(b-1)		$\frac{MsAB}{MsAB \times Ss}$
AB x S	SS within S_s - [SS_A + SS_B + SS_{AB} + $SS_A \times S_s$ + $SS_B \times S_s$]	(a-1)(b-1)(n-1)		

II. Two factor ANOVA with repeated measures on each factor for the periods of 12-hour access and following 12-hour access in dynamic VMH-lesioned rats fed milk

Subjects	12-hour access			
	Day 1	2	3	4
LC1	23	29	17	32
LC2	25	39	42	42
LC4	39	31	36	38
LC13	39	44	41	37
n				
Σx	126	143	136	149
$\bar{x}$	31.5	35.8	34.0	37.3
n $\Sigma(x)^2$	4196	5259	5030	5601
	24-hour access			
LC1	29	38	34	15
LC2	52	38	43	41
LC4	42	38	52	46
LC13	50	44	50	66
n	173	158	179	168
Σx	43.3	39.5	44.8	42.0
$\bar{x}$				
n $\Sigma(x)^2$	7809	62 68	8209	8378
an $\Sigma\Sigma x$	299	301	315	317

$\frac{bn}{\Sigma\Sigma x} = 554$

$\bar{x} = 34.62$

$\frac{bn}{\Sigma\Sigma x} = 678$

$\bar{x} = 42.40$

$\frac{abn}{\Sigma\Sigma\Sigma x} = 1232$

$\frac{abn}{\Sigma\Sigma\Sigma(x)^2} = 50750$

SS total

$$50750 - \frac{(1232)^2}{32} = 3318.0$$

SS between Ss

$$\frac{392,098}{8} - \frac{1517824}{32} = 1580.25$$

SS within Ss

$$3318.00 - 1580.25 = 1737.75$$

SS_A

$$\frac{(554)^2 + (678)^2}{16} - \frac{1517824}{32} = 480.50$$

$SS_A \times S$

$$\frac{198278}{4} - \frac{392098}{8} - \frac{76600}{16} + \frac{1517824}{32} = 76.75$$

SS_B

$$\frac{379716}{8} - \frac{1517824}{32} = 32.50$$

$SS_B \times Ss$

$$\frac{98652}{2} - \frac{392098}{8} - \frac{379716}{8} + \frac{1517824}{32} = 281.20$$

SS_{AB}

$$\frac{192180}{4} - \frac{379716}{8} - \frac{766600}{16} + \frac{1517824}{32} = 100.00$$

$SS_{AB} \times Ss$

$$1737.75 - [408.50 + 32.50 + 100.00 + 76.75 + 281.25] = 766.75$$

For ANOVA source table, see table 18.

III. Two Factor ANOVA with repeated measures on each factor for the periods of 12-hour access and following 12-hour access in dynamic VMH-lesioned rats fed chow.

Subjects	12-hour access			
	Days[1]			
	1	2	3	
LC14	22.7	25.8	30.5	
LC16	21.9	22.1	21.9	
LC20	20.5	33.0	31.1	
LC24	28.0	24.8	36.7	
LC26	36.1	44.4	45.7	
LC28	27.2	31.4	34.2	
LC32	23.1	26.5	30.2	
n				bn
$\underline{\Sigma x}$	179.5	208.0	230.3	$\Sigma\Sigma x = 617.5$
x	25.6	29.7	32.9	
n				
$\Sigma(x)^2$	4775.81	6517.66	7894.13	$\bar{x} = 29.4$
	24-hour access			
	Days			
	1	2	3	
LC14	38.7	37.3	42.1	
LC16	23.0	26.2	30.7	
LC20	32.1	28.1	27.0	
LC24	39.8	43.8	38.0	
LC26	50.2	52.4	57.6	
LC28	43.0	35.2	37.8	
LC32	40.5	50.6	47.1	
n				
$\underline{\Sigma x}$	267.3	273.6	280.3	
x	38.2	39.1	40.0	$\bar{x} = 39.1$
n				
$\Sigma(x)^2$	10650.43	11330.94	11852.91	bn $\Sigma\Sigma x = 821.2$
				abn $\Sigma\Sigma\Sigma x = 1438.7$
				abn $\Sigma\Sigma\Sigma(x)^2 = 53021.88$

[1]Since only 3 days' data were collected in some animals only the first 3 days in each condition were used.

SS total

$$53{,}021.88 - \frac{(1438.7)^2}{42} = 3739.554$$

SS between S_s

$$\frac{307{,}330.90}{6} - \frac{2{,}069{,}857.6}{42} = 1939.49$$

SS within S_s

$$3739.554 - 1939.49 = 1800.064$$

SS_A

$$\frac{(617.5)^2 + (821.2)^2}{21} - \frac{2{,}069{,}857.6}{42} = 987.945$$

$SS_A \times S$

$$\frac{157{,}598.84}{3} - \frac{30{,}7330.9}{6} - \frac{1{,}055{,}675.6}{21} + \frac{2{,}069{,}857.6}{42} = 323.186$$

SS_B

$$\frac{692{,}281.16}{14} - \frac{2{,}069{,}857.6}{42} = 166.328$$

$SS_B \times s$

$$\frac{102{,}897.54}{2} - \frac{30{,}7330.9}{6} - \frac{692{,}281.16}{14} + \frac{2{,}069{,}857.6}{42} = 60.626$$

SS_{AB}

$$\frac{353{,}396.68}{7} - \frac{692{,}281.16}{14} - \frac{1{,}055{,}675.6}{21} + \frac{2{,}069{,}857.6}{42} = 48.64$$

$SS_{AB} \times S_s$

$$1800.06 - [\, 987.945 + 166.328 + 48.64 + 323.186 + 60.626 \,] = 213.338$$

For ANOVA source table, see table 19.

IV. Two factor ANOVA with repeated measures on each factor for the 12-hour access and following 12-hour access in dynamic VMH-lesioned rats fed HF diet.

Subjects	12-hour access				
	Day[1]				
	1	2	3	4	
LC38	28.5	33.3	34.3	37.3	
LC40	28.6	27.9	34.9	29.2	
LC44	19.2	23.8	26.4	24.9	
LC36	26.3	35.2	35.0	28.7	
LC34	20.5	18.7	28.7	32.6	
n					
Σx	123.10	138.9	159.3	152.7	
$\bar{x}$	24.6	27.8	31.9	30.5	$\bar{x} = 28.7$
n					
$\Sigma(x)^2$	3110.79	4042.47	5140.15	4750.39	bn $\Sigma\Sigma x = 574$
	24-hour access				
	Day				
	1	2	3	4	
LC38	37.2	27.9	38.2	45.9	
LC40	33.4	36.0	36.7	39.9	
LC44	25.9	27.9	28.0	29.4	
LC36	29.5	34.9	43.6	24.5	
LC34	28.8	41.9	35.2	21.0	
n					
Σx	154.8	168.6	181.7	160.7	
$\bar{x}$	31.0	33.7	36.3	32.1	$\bar{x} = 33.5$
n					
$\Sigma(x)^2$	4869.9	5826.44	6730.13	5604.4	bn $\Sigma\Sigma x = 665.8$
					abn $\Sigma\Sigma\Sigma x = 1239.8$
					abn $\Sigma\Sigma\Sigma(x)^2 = 40074.7$

[1]Since only 4 days' data were collected in some animals only the first 4 days in each condition were used.

SS total

$$40074.70 - \frac{(1239.8)^2}{40} = 1{,}647.099$$

SS between

$$\frac{311185.58}{8} - \frac{1537104.04}{40} = 470.5965$$

SS within

$$1647.099 - 470.5965 = 1176.5025$$

SS_A

$$\frac{(574)^2 + (665.8)^2}{20} - \frac{1537104.04}{40} = 210.681$$

$SS_A \times S_s$

$$\frac{156609.64}{4} - \frac{311185.58}{8} - \frac{772765.64}{20} + \frac{1537104.04}{40} = 43.531$$

SS_B

$$\frac{386285.22}{10} - \frac{1537104.04}{40} = 200.921$$

$SS_B \times S$

$$\frac{78778.62}{2} - \frac{311185.58}{8} - \frac{386285.22}{10} + \frac{1537104.04}{40} = 290.1915$$

SS_{AB}

$$\frac{194368.98}{5} - \frac{386285.22}{10} - \frac{772765.64}{20} + \frac{1537104.04}{40} = 34.593$$

$SS_{AB} \times s$

$$1176.5025 - [210.681 + 200.921 + 34.593 + 43.531 + 290.1915] = 396.584$$

For ANOVA source table, see Table 20.

V. Two-factor ANOVA with repeated measures on each factor for comparing intakes on the days prior to the second restriction and immediately following the second restriction with chow

Subjects	24-hour access before restriction				
	Day[1]				
	1	2	3	4	
LC14	22.2	23.2	27.5	25.3	
LC16	33.0	30.4	33.9	32.7	
LC20	20.8	28.2	23.5	35.5	
LC24	27.2	28.5	25.3	26.1	
LC26	32.4	32.6	22.7	27.7	
LC28	34.0	28.6	25.0	24.7	
LC32	24.3	24.8	29.4	27.6	$\Sigma\Sigma x = 777.1$
n					
Σx	193.9	196.3	187.3	199.6	
$\bar{x}$	27.7	28.0	26.8	28.5	
n					
$\Sigma(x)^2$	5550.57	5565.65	5102.45	5789.98	$\bar{\bar{x}} = 27.7$
	24 hour access				
	Day				
	1	2	3	4	
LC14	20.2	24.1	21.7	29.8	
LC16	18.0	28.0	24.2	31.3	
LC20	25.0	18.4	28.2	25.4	
LC24	17.8	20.5	21.5	24.4	
LC26	21.0	33.0	30.7	31.0	
LC28	28.3	25.9	28.3	27.5	
LC32	23.4	23.4	26.0	28.9	
n					
Σx	153.7	173.3	180.6	198.3	$\bar{\bar{x}} = 25.2$
$\bar{x}$	22.0	24.8	25.8	28.3	
n					
$\Sigma(x)^2$	3463.33	4430.99	4733.40	5660.71	$\Sigma\Sigma x = 705.9$

$\Sigma\Sigma\Sigma x = 1483.0$

$\Sigma\Sigma\Sigma(x)^2 = 40,297.08$

[1]Since only 4 days' data were collected in some animals only the 4 days immediately before and the 4 days immediately after the restriction are considered.

SS total

$$40,297.08 - \frac{(1483.0)^2}{56} = 1024.062$$

SS between subjects

$$\frac{315,854.28}{8} - \frac{(2199289)}{56} = 208.767$$

SS within

$$1024.062 - 208.77 = 815.295$$

SS_A

$$\frac{(771.1)^2 + (705.9)^2}{28} - \frac{2199289}{56} = 90.525$$

$SS_A \times S_s$

$$\frac{158,671.22}{4} - \frac{315,854.28}{8} - \frac{1,102,179.2}{28} + \frac{2199289}{56} = 95.495$$

SS_B

$$\frac{551,104.74}{14} - \frac{2,199,289}{56} = 91.606$$

$SS_B \times S_s$

$$\frac{79504.72}{2} - \frac{315,854.28}{8} - \frac{51,104.74}{14} + \frac{2,199,289}{56} = 178.969$$

SS_{AB}

$$\frac{276,648.18}{7} - \frac{551,104.74}{14} - \frac{1,102,179.2}{28} + \frac{2,199,289}{56} = 66.404$$

$SS_{AB} \times S_s$

$$815.295 - [90.525 + 91.606 + 66.404 + 95.495 + 178.969] = 292.295$$

For ANOVA source table see table 21

VI Two-factor ANOVA with repeated measures on each factor for comparing intakes on the days prior to the second restriction and immediately following the second restriction with the HF diet

Subjects	24-hour access before restriction			
	Day[1]			
	1	2	3	
LC38	22.3	25.4	20.7	
LC40	22.2	13.8	19.6	
LC44	25.0	25.2	22.3	
LC36	26.7	26.0	29.4	
LC34	28.7	27.3	27.6	
n				
Σx	124.9	117.7	119.6	$\bar{x} = 24.1$
$\bar{x}$	25.0	23.5	23.9	
n				
$\Sigma(x)^2$	3151.71	2891.93	2936.06	bn $\Sigma\Sigma x = 362.2$
	24-hour access following restriction			
	Day			
	1	2	3	
LC38	16.1	16.2	17.0	
LC40	16.8	14.5	16.7	
LC44	12.4	15.3	19.4	
LC36	20.0	12.9	16.8	
LC34	25.9	27.4	22.6	
n				
Σx	91.2	86.3	92.5	$\bar{x} = 27.0$
$\bar{x}$	18.2	17.3	18.5	
n				
$\Sigma(x)^2$	1766.02	1623.95	1737.25	bn $\Sigma\Sigma x = 270.0$
				abn $\Sigma\Sigma\Sigma = 632.2$
				abn $\Sigma\Sigma\Sigma(x)^2 = 14106.92$

[1]Since only 3 days' data were collected in some animals, only the 3 days immediately before and the 3 days immediately following the restriction are considered.

SS total

$$14{,}106.92 - \frac{(632.2)^2}{30} = 784.36$$

SS between subjects

$$\frac{81{,}701.9}{6} - \frac{399{,}676.84}{30} = 294.422$$

SS within

$$784.36 - 294.422 - 489.94$$

SS_A

$$\frac{(362.2)^2 + (270.0)^2}{15} - \frac{399{,}676.84}{30} = 283.361$$

$SS_A \times S_s$

$$\frac{41{,}939.34}{3} - \frac{81{,}701.9}{6} - \frac{204{,}088.84}{15} + \frac{399{,}676.84}{30} = 79.436$$

SS_B

$$\frac{133301.620}{10} - \frac{399{,}676.84}{30} = 7.601$$

$SS_B \times S_s$

$$\frac{27{,}365.72}{2} - \frac{81{,}701.9}{6} - \frac{13301.620}{10} + \frac{399{,}676.84}{30} = 58.276$$

SS_{AB}

$$\frac{68{,}078.84}{5} - \frac{133{,}301.62}{10} - \frac{204{,}088.84}{15} + \frac{399{,}676.84}{30} = 2.245$$

$SS_{AB} \times S_s$

$$489.94 - [283.361 + 7.601 + 2.245 + 79.436 + 58.276] = 59.021$$

For ANOVA source table, see table 22.

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