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Virgil Charles Nylander
Iowa State University

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Olfactory and palatability factors regulating food intake in VMH lesioned rats

by

Virgil Charles Nylander

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INTRODUCTION

Finickiness is an attribute commonly used to describe the feeding behavior of ventromedial hypothalamic (VMH) lesioned rats. They are characterized as being overly-sensitive to both palatable and unpalatable diets, consuming more palatable and less unpalatable food than controls (Corbit & Stellar, 1964; Miller, Bailey, & Stevenson, 1950; Panksepp, 1971). Studies which have reported these observations, however, often fail to consider the influence of the hyperphagic rat's obesity on its intake of diets which vary along a palatability dimension. VMH lesioned rats respond to unpalatable food in a manner quite analogous to controls as long as they are not allowed to become obese prior to palatability testing. Under these conditions VMH lesioned rats do not over-eat unpalatable diets, but neither do they undereat (Ferguson & Keesey, 1975; Franklin & Herberg, 1974; Sclafani & Kluge, 1974; Sclafani, Springer, & Kluge, 1976).

Sclafani (1976) has recently revised a dual lipostatic model of hunger and appetite (Sclafani & Kluge, 1974) which attempts to specify the brain mechanisms that regulate feeding behavior. The model proposes the existence of two separate lipostatic mechanisms that control the lower and upper limits of an animal's body weight. The mechanism that controls the lower limit of body weight stimulates hunger when the animal's body weight falls below a fixed set point. "Hunger is defined here as the drive for food aroused by internal stimuli which accumulate during food deprivation and body weight

loss" (Sclafani, 1976, P. 281). The mechanism that controls the upper body weight limit operates by inhibiting the animal's appetite as its body weight exceeds the set point. Appetite "is defined as the incentive to eat aroused by external food-related stimuli, e.g., taste" (Sclafani, 1976, P. 281). Normal body weight, according to this model, should therefore vary around a single set point depending upon diet palatability.

This model postulates that hyperphagia produced by VMH lesions is the result of damage to the upper lipostatic mechanism. Damage to this mechanism results in a disinhibition of the appetite controlling mechanism. The VMH lesioned animal overeats because the food remains palatable due to the reduction of appetite inhibiting signals from the upper lipostatic mechanism. The VMH lesioned animal will continue to eat "until its weight is sufficiently above the set point such that the inhibitory output of the upper lipostat equals the excitatory effect of the diet" (Sclafani, 1976, P. 287).

The palatability of a diet is determined by several of its qualities such as taste and texture. One of these qualities, odor has received scant experimental attention. In a review of olfaction and nutrition Le Magnen (1971) stated: "As a result of innate responses modified by experience, the odor of foods contributes to the current oral sensory control of food intake. This control promotes a regulation because metabolic influences are both current and delayed. The onset and size of the oral

feeding response are dependent on the present state of food deprivation or hunger. On the other hand, postabsorptive repleting effects reinforce and regulate the metering action of this oral control" (P. 475). Further support for olfactory involvement in the control of food intake and palatability is offered by Pager, Giachetti, Holley, and Le Magnen (1972). They found, in food deprived rats, positive electrical activity in the mitral cell layer of the olfactory bulb to the odor of their regular stock diet. Negative or null responses were obtained when the satiated animals were exposed to their regular diet or to control odors. The results suggest that the state of food deprivation is involved in either facilitating or inhibiting the activity of the olfactory bulbs.

Interactions between olfactory and hypothalamic mechanisms in the control of food intake have been supported by the demonstration of neurological connections between these structures (MacLeod, 1971; Scott & Leonard, 1971; Scott & Pfaffmann, 1967). The recording of electrical activity in the VMH to olfactory stimuli has added further support to the possible importance of these interactions in the control of food intake (Campbell, Bindra, Krebs, & Ferenchak, 1969).

A major technique used to investigate the role of olfaction in the regulation of food intake, as well as a number of other behaviors, is ablation of the olfactory bulbs. The effect of bulbectomy on food intake was investigated by Larue and Le Magnen

(1972). They found that bulbectomized rats did not differ from sham operated controls in their mean daily food intake. However, they did notice that there was more variability in daily food intake among individual bulbectomized rats than among controls. Further examination revealed that the feeding pattern consisted of successive and small periods of eating separated by many short rest periods. This pattern of feeding behavior is referred to as "nibbling" and has also been found in recovered lateral hypothalamic (LH) lesioned rats (Kisseleff, 1970).

Larue and Le Magnen (1972) contend that the nibbling pattern of food intake following bulbectomy is probably the result of interruption of olfacto-hypothalamic pathways. They state that reduction of food palatability due to suppression of its odorous stimulating activity results in loss of rewarding information which is essential for maintenance of large meals. A similar position is held by Cain (1974) who suggests that the olfactory bulb is involved in a forebrain arousal mechanism as well as in olfaction. He supports a model similar to the two-arousal hypothesis proposed by Routtenberg (1968 & 1971). This hypothesis states that the olfactory mechanism is part of a limbic midbrain system which is primarily concerned with incentive or reward. Destruction of the olfactory bulbs is assumed to disrupt normal functions relating to reinforcement. Support for the reinforcing component of the olfactory system is found in the observation that electrical self-stimulation is readily obtained from olfactory areas (Routtenberg,

1971).

In an attempt to determine the effects of impaired orosensory cues on food intake in VMH lesioned rats Larue and Le Magnen (1970) performed bulbectomies on VMH lesioned rats. One group of VMH lesioned rats received bulbectomies as soon as they had attained their asymptotic weight levels. A second group which had attained their asymptotic weight level was reduced to preoperative weight levels before being bulbectomized. Both groups of bulbectomized VMH lesioned rats exhibited an increased period of hyperphagia and an appreciably higher final asymptotic weight level. These were unanticipated results, because a reduction in the peripheral sensory input and thus a decrease in food palatability should have produced a decrease in food intake. These results were interpreted in terms of oral intake becoming more dependent on unadjusted responses to the palatability of the food.

The results of the previous study as well as others using the bulbectomy technique have recently been criticized (Alberts, 1974; Cain, 1974). Once a bulbectomy is performed it is often assumed that the behavioral outcome is the result of loss of the sense of smell. This assumption unfortunately does not take into consideration the numerous connections which the olfactory bulbs have with other central nervous system (CNS) structures (Alberts, 1974). The number of CNS connections and the diverse behavioral responses resulting from bulbectomies suggest a much more integra-

tive and important role for olfactory structures. Therefore, it appears that bulbectomies not only interfere with olfaction but also with other CNS functions.

A technique for the elimination of olfaction without damaging the olfactory bulb was reported by Alberts and Galef (1971). They found that the application of a 5% solution of zinc sulfate ($ZnSO_4$) to the nasal epithelium produced cellular degeneration of the olfactory receptors. This deficit lasts between 5-7 days before the occurrence of cellular regeneration and the return of olfactory sensations.

The use of zinc sulfate ($ZnSO_4$) treatment to impair olfactory function has resulted in reversals of a number of earlier findings: increased irritability and mouse killing in bulbectomized rats are absent when zinc sulfate is utilized (Alberts & Friedman, 1972; Cain & Paxinos, 1974); abnormal forms of maternal behavior observed after bulbectomy were not present with zinc sulfate treated subjects (Fleming & Rosenblatt, 1974); and preliminary evidence indicates that zinc sulfate treated subjects do not differ from controls in their pattern of daily food intake (Cain, 1974). Because of these reversals, the results of studies utilizing bulbectomies as a technique to eliminate an organism's olfactory capabilities must be re-examined.

The purpose of the present experiment was to determine the effects of olfactory and palatability factors on food intake and body weight in VMH lesioned rats. It was hypothesized that food

intake and body weight are determined, in part, by the palatability of a diet. Therefore, any decrease in palatability due to an olfactory deficit should produce a corresponding decrease in body weight and food intake with highly palatable foods. Similarly when fed an unpalatable diet, the VMH lesioned rat with an olfactory deficit should consume more food and lose less body weight than a VMH lesioned rat without an olfactory deficit. This palatability hypothesis was tested using two 24-hour testing periods during which all animals had free access to a palatable high-fat diet during one of the testing periods and an unpalatable quinine-adulterated diet during the second testing period. Food intake and body weight measures were taken following each testing period.

Olfactory deficits in VMH lesioned and control rats were produced using two different techniques. A peripherally induced anosmic state was produced by the $ZnSO_4$ treatment developed by Alberts and Galef (1971). Recent evidence has indicated that this treatment has a 20% mortality rate (Mayer & Rosenblatt, 1975); therefore, each rat experienced only two treatments.

The second technique used to produce an olfactory deficit was bulbectomy. If a bulbectomy only destroys structures related to olfaction, there should be no differences between the food intake and body weight of $ZnSO_4$ treated and bulbectomized VMH lesioned subjects during the 24-hour testing periods. If, however, the bulbectomized rats consummatory behavior is different than $ZnSO_4$ treated rats, then neural mechanisms other than those involved

in olfaction are disturbed by the bulbectomy. This outcome was a possibility since Larue and Le Magnen (1970) have previously demonstrated that VMH bulbectomized rats consume more than VMH controls. The effects of olfactory deficits on long term food intake of both palatable and unpalatable diets were also determined by initially providing all rats with an unrestricted amount of butyric acid-adulterated food for 18 days. This provided a partial replication of the procedures used by Ferguson and Keesey (1975), wherein they found that VMH lesioned rats respond almost identically to controls in maintaining body weight when fed an unpalatable diet. Free access to the palatable high-fat diet for 60 days provided further information concerning the effects of olfactory deficits on long term food intake. The final phase of this experiment was also a partial replication of the procedures used by Larue and Le Magnen (1970), who found increased body weight gains in VMH bulbectomized rats.

METHOD

Subjects

The subjects were 80 adult Long-Evans female hooded rats (Blue Spruce Farms, Inc.). The rats weighed between 207 and 284 grams at the beginning of the experiment. They were housed individually in wire-mesh cages and were provided with continuous illumination in a temperature controlled (23° C) room.

Diets

All rats received three diets during the course of the experiment; a high-fat diet, a quinine-adulterated diet, and a butyric acid-adulterated diet. The high-fat diet consisted of two parts by weight ground chow (Teklad Mouse and Rat Diet) and one part by weight melted Crisco brand hydrogenated vegetable shortening. The quinine-adulterated diet consisted of seven parts by weight water and three parts by weight ground chow adulterated with quinine sulfate (.20% by weight). The butyric acid-adulterated diet also consisted of seven parts water and three parts ground chow, but was adulterated with N-butyric acid (7.0% by weight). The diets were presented in 8-ounce glass ointment jars attached to the front of each cage. Water was available *ad libitium* throughout the experiment.

Surgery

Rats were anesthetized with Myothesia (42 mg/kg) prior to receiving bilateral electrolytic lesions aimed at the VMH.

Stereotaxic coordinates (de Groot, 1959) for the VMH lesions were: 5.8 mm anterior, .7 mm lateral, and .5 mm above the floor of the skull. The lesions were produced by passing 2.0 ma anodal current for 20 seconds between a 30-gauge nichrome steel electrode, insulated except at its conical tip, and a rectal cathode. All rats not lesioned received sham operations which were identical to VMH surgeries except that the electrode was not lowered.

Rats receiving bilateral bulbectomies were also anesthetized with Myothesia and the olfactory bulbs were removed by suction through a perforation of the skull. The cribriform plate was scraped to insure a complete separation of peripheral and central olfactory structures and the remaining cavity was loosely packed with gelfoam. Those rats not receiving bulbectomies received the same surgical treatment except that the olfactory bulbs were not aspirated through the skull perforation.

Procedure

Prior to surgery all rats were allowed free access to the high-fat diet for seven days. Daily food intake and body weight measures were used to establish preoperative baselines. Body weight and food intake baselines were defined as the mean of the four days preceding surgical preparation. All rats were maintained at their baseline body weights throughout the experiment unless otherwise indicated.

Rats randomly assigned to VMH and non-VMH groups were deprived of food and water for 24 hours preceding and following surgery.

After the 24-hour postoperative period all rats were allowed free access to the high-fat diet for a period not exceeding 13 days. During this period food intake and body weights were recorded daily as a preliminary screening procedure to identify hyperphagic VMH rats prior to subsequent bulbectomy surgery. Lesioned rats were initially classified as hyperphagic on the basis of substantial increments in body weight and food intake relative to control measures. When a lesioned rat was classified as hyperphagic, it and a control rat were reduced to their preoperative body weights using reduced portions of the high-fat diet. VMH lesioned rats not classified as hyperphagic at the end of this 13 day period were eliminated from the experiment and the remaining control rats were reduced to preoperative weight levels.

Rats were randomly assigned to one of six groups following the screening procedure. The six groups were as follows: control-$ZnSO_4$ (C-Zn), VMH-$ZnSO_4$ (VMH-Zn), control-saline (C-S), VMH-saline (VMH-S), control-bulbectomy (C-B), and VMH-bulbectomy (VMH-B). Fifteen days following VMH surgery rats in the C-B and VMH-B groups received bulbectomies and all other rats received sham bulbectomies. A seven day recovery period followed this second surgery during which all rats were maintained at their preoperative weights using the high-fat diet.

Following the seven day recovery period rats in the C-Zn and VMH-Zn groups received $ZnSO_4$ treatments and all other rats received saline treatments. Rats were anesthetized with ether prior to the

treatments. A forcep was then attached to the tip of the tongue to displace it from obstructing the passage of a curved 20-gauge needle constructed to the specification described by Alberts and Galef (1971). The needle was inserted into the rat's mouth until the tip entered the nasal cavity through the posterior choanae. The rat was turned upside down and either .8 cc of a 5% $ZnSO_4$ solution dissolved in physiological saline or physiological saline was injected into the nasopharyngeal cavity. The needle was removed and the rat was held upside down allowing the solution to run out the external nares. The rat was held in this position until the rat regained consciousness.

To insure that possible aversive side effects of the injections had subsided, a three day period elapsed between the treatment and olfactory discrimination and palatability tests. The olfactory discrimination test was used to evaluate the rat's ability to perceive an odorous substance and thus provide an indication of the effectiveness of the $ZnSO_4$ and bulbectomy treatments. For the olfactory test, similar to that used by Cain and Paxinos (1974), four small metal cylindrical containers (water-proof match containers) were suspended from the ceiling of each rat's home cage. Each cylinder's upper half was perforated with holes to allow the passage of odor through the cylinder. One of the metal cylinders contained a mixture of water and chocolate chip cookies, the other three cylinders were empty. Pieces of chocolate chip cookies had been presented to each rat prior to the testing

session to insure that the odor associated with the cookies would not be novel. The cookie mash was not visible in the container and the position of the cookie mash container was randomly varied each trial. Two five-minute trials were given during each discriminations test. The amount of time the rat's nose was within approximately one cm of either the cookie-containing cylinder or any of the other three cylinders was recorded with two electronic timers.

Two palatability tests were used to assess the effects of deficits in olfactory function on food intake. Because the $ZnSO_4$ deficits are variable and relatively short term, a first 24-hour test was given three days after the treatment for all rats. One-half of the rats in each of the six groups received free access to the high-fat diet and the remaining rats received the quinine-adulterated diet. The amount of food consumed and changes in body weight were measured at the completion of the testing period. The rats were then returned to their preoperative weights. All rats received identical $ZnSO_4$ or saline treatments three days after the first palatability test and a second olfactory and palatability test occurred three days post-treatment. During the second palatability test each rat received the diet not presented during the first testing period.

Following the second palatability test all rats were returned to their preoperative weights and were then given free access to the butyric acid-adulterated diet for 18 days. All rats were then

fed the high-fat diet for 15 days. These procedures were used to assess possible long term effects of the olfactory manipulations ($ZnSO_4$ and bulbectomy) in both lesioned and control rats fed palatable and unpalatable diets. The VMH lesioned rats were then maintained on the high-fat diet for an additional 48 days to determine if asymptotic body weights were a function of the olfactory manipulations.

At the conclusion of the 48 days all VMH lesioned rats were again given the olfactory discrimination test. This test was given to determine the degree of olfactory impairment in both $ZnSO_4$ and bulbectomized rats after a prolonged time period.

Histology

All VMH lesioned and/or bulbectomized rats were given an overdose of sodium pentobarbital at the conclusion of the experiment. They were perfused intracardially with physiological saline and 10% formalin. The brains of all VMH lesioned rats were removed and frozen sections were taken at 150μ. Photographic enlargements of the unstained sections were used to assess the location and extent of damage. Bulbectomized rat brains were visually examined to determine the extent of damage to the olfactory bulbs and to verify the separation of the bulbs from their peripheral connections.

RESULTS

Fifty-one of 80 rats completed the study. The 29 rats that failed to complete the study were eliminated for the following reasons: 4 rats died during surgery, 17 rats were discarded for failure to pass an initial VMH screening test, 3 rats died during $ZnSO_4$ treatments, and 5 rats were discarded following histological examination.

Olfactory Discrimination Test

Mean percent "sniffing times" for each group during each of the olfactory discrimination test sessions are presented in Table 1. Since the cookie-mash was randomly placed in one of four containers, the mean percent sniffing time for rats with impaired olfactory capabilities should approximate chance, i.e., 25 percent. Olfactory function was considered unimpaired if rats spent 50 percent of their sniffing time at the cookie-mash container. The data indicate that all of the olfactory impaired rats spent approximately 25 percent of their sniffing time at the cookie-mash containers during the first two test sessions. The third test session, which took place 81 days following the last $ZnSO_4$ procedure, demonstrates that the VMH-Zn group had regained their olfactory capabilities and responded in a manner similar to that of the VMH-S group. The third test session also provides further behavioral evidence that the VMH-B group was still deficient in olfactory function. It was not necessary to discard a single animal from any group receiving either bulbectomy or $ZnSO_4$ treatments since no score exceeded

Table 1

Mean Percent Sniffing Time During Olfactory Discrimination Tests

		TEST SESSIONS		
Group	n Tested	1	2	3
C-S	9	65.5	59.8	
C-Zn	8	22.6	28.4	
C-B	12	26.1	24.9	
VMH-S	6	71.2	62.8	68.1
VMH-Zn	9	27.4	24.3	59.7
VMH-B	7	25.1	23.7	28.4

50 percent.

Palatability Test

Results of the two 24-hour palatability tests are found in Tables 2 and 3. Body weight changes were analyzed using analysis of covariance. The covariate was the rat's body weight preceding palatability testing. The results indicate a significant difference ($F = 26.29$, $df = 1/44$, $p < .001$) between VMH lesioned and nonlesioned rats on the amount of weight gained during the high-fat palatability test (Table 4). A significant difference in body weight ($F = 3.97$, $df = 2/44$, $p < .05$) was also found between olfactory treatments during the quinine-adulterated palatability test (Table 5).

Table 2

Mean Change in Body Weight (Grams) Following

24-Hour Palatability Tests

		High-Fat		Quinine	
Group	n Tested	$\bar{X}$	S	$\bar{X}$	S
C-Zn	8	13.13	3.91	-5.25	4.03
C-S	9	16.11	5.18	-2.78	4.12
C-B	12	19.67	5.60	-7.58	3.37
VMH-Zn	9	28.33	9.33	-2.89	4.34
VMH-S	6	23.50	6.28	-3.67	1.21
VMH-B	7	28.00	10.05	-5.86	2.12

Table 3

Mean Food Intake (Grams) During Palatability Testing

		High-Fat		Quinine	
Group	n Tested	$\bar{X}$	S	$\bar{X}$	S
C-Zn	8	19.38	2.83	18.13	3.83
C-S	9	20.56	2.55	32.00	12.61
C-B	12	22.08	4.44	15.75	10.35
VMH-Zn	9	28.11	6.07	16.56	4.16
VMH-S	6	24.67	1.97	18.00	4.60
VMH-B	7	27.14	5.87	19.71	21.26

Table 4

Analysis of Covariance Source Table for 24-Hour High-Fat Palatability Test Body Weight Data

Source	SS	df	MS	F	p
VMH Lesion (A)	1293.68	1	1293.68	26.29	.001
Olfactory Treatments (B)	72.67	2	72.67	1.48	
A x B	72.79	2	72.79	1.48	
Within Cell	49.20	44	49.20		

Table 5

Analysis of Covariance Source Table for 24-Hour Quinine-Adulterated Palatability Test Body Weight Data

Source	SS	df	MS	F	p
VMH Lesion (A)	17.67	1	17.67	1.43	
Olfactory Treatments (B)	97.80	2	48.90	3.97	.05
A x B	28.57	2	14.29	1.16	
Within Cell	542.02	44	12.32		

A Tukey's post-hoc multiple comparisons test indicated that the bulbectomized group lost significantly more weight on the quinine-adulterated diet than the saline treated group ($p < .05$).

Analysis of variance was performed on the food intake data for only the high-fat palatability tkst (Table 6). A significant difference ($F = 23.05$, $df = 1/45$, $p < .001$) between VMH lesioned and nonlesioned rats was found. This indicates that VMH lesioned rats consumed significantly more than nonlesioned rats. Analysis of the food intake data for the quinine-adulterated diet was not performed because of large amounts of food spillage.

Table 6

Source Table for 24-Hour High-Fat Food Intake Palatability Test

Source	SS	df	MS	F	p
VMH Lesion (A)	433.37	1	433.37	23.05	.001
Olfactory Treatments (B)	33.08	2	16.54	.88	
A x B	47.97	2	23.98	1.27	
Within Cell	846.09	45	18.80		

The overall results of the 24-hour palatability test indicate that VMH lesioned rats tend to eat more and gain more weight on

the highly palatable high-fat diet than do nonlesioned rats. This difference between VMH lesioned and nonlesioned rats was not found with the unpalatable quinine-adulterated diet. The only apparent effect of olfactory manipulations was a greater loss in weight for the bulbectomized than for the saline treated rats on the quinine-adulterated diet.

Butyric Study

Body weight and food intake were recorded during each of the 18 days of the butyric study (Appendix, Tables A and B). Daily body weights and food intake were regressed on orthogonal polynomials to obtain intercept, linear, and quadratic coefficients. Between group comparisons were made for: (A_1) VMH lesion versus nonlesion; (B_1) $ZnSO_4$ treatment versus bulbectomy; (B_2) saline treatment versus $ZnSO_4$ treatment and bulbectomy.

Analysis of the intercept, linear, and quadratic coefficients for body weight and food intake are found in Tables 7 and 8. The results indicate a significant linear coefficient for body weight between VMH lesioned and nonlesioned rats. This indicates a steeper decreasing regression line slope for VMH lesioned than for nonlesioned rats. The VMH lesioned rats lost more body weight over the 18 day period than did nonlesioned rats.

Food intake analysis demonstrates significant intercept and quadratic coefficients between VMH lesioned and nonlesioned rats. The significant intercept indicates that the VMH lesioned rats ate less food than did nonlesioned rats when food intake was

Table 7

Analysis of the Intercept, Linear, and Quadratic Coefficients for Body Weight During the Butyric Acid-Adulterated Diet Test

	Source	SS	df	MS	F
Intercept	VMH Lesion (A_1)	0.08	1	0.08	0.00
	$ZnSO_4$ vs. Bulbectomy (B_1)	17.74	1	17.74	0.05
	Saline vs. $ZnSO_4$ & Bulb. (B_2)	96.79	1	96.79	0.30
	$A_1 \times B_1$	94.49	1	94.49	0.29
	$A_1 \times B_2$	131.86	1	131.86	0.40
	Error	14753.33	45	327.85	
	Corrected Total	15098.51	50		

	VMH Lesion (A_1)	1.362	1	1.362	7.41*
	$ZnSO_4$ vs. Bulbectomy (B_1)	0.046	1	0.046	0.22
	Saline vs. $ZnSO_4$ & Bulb. (B_2)	0.004	1	0.004	0.02
Linear	$A_1 \times B_1$	0.032	1	0.032	0.15
	$A_1 \times B_2$	0.011	1	0.011	0.05
	Error	9.558	45	.212	
	Corrected Total	11.492	50		
	VMH Lesion (A_1)	0.0000	1	0.0000	0.00
	$ZnSO_4$ vs. Bulbectomy (B_1)	0.0018	1	0.0018	1.58
	Saline vs. $ZnSO_4$ & Bulb. (B_2)	0.0015	1	0.0015	1.33
Quadratic	$A_1 \times B_1$	0.0003	1	0.0003	0.23
	$A_1 \times B_2$	0.0021	1	0.0021	1.76
	Error	0.0519	45	0.0012	
	Corrected Total	0.0592	50		

*$p < 0.01$

Table 8

Analysis of the Intercept, Linear, and Quadratic Coefficients for Food Intake During the Butyric Acid-Adulterated Diet Test

	Source	SS	df	MS	F
Intercept	VMH Lesion (A_1)	275.08	1	275.08	12.61*
	$ZnSO_4$ vs. Bulbectomy (B_1)	19.79	1	19.79	0.91
	Saline vs. $ZnSO_4$ & Bulb. (B_2)	0.235	1	0.235	0.01
	$A_1 \times B_1$	6.43	1	6.43	0.29
	$A_1 \times B_2$	1.57	1	1.57	0.07
	Error	981.68	45	21.82	
	Corrected Total	1459.42	50		

	VMH Lesion (A_1)	0.055	1	0.055	2.09
	$ZnSO_4$ vs. Bulbectomy (B_1)	0.163	1	0.163	6.26*
	Saline vs. $ZnSO_4$ & Bulb. (B_2)	0.053	1	0.053	2.01
Linear	$A_1 \times B_1$	0.014	1	0.014	0.52
	$A_1 \times B_2$	0.038	1	0.038	1.46
	Error	1.175	45	0.026	
	Corrected Total	1.510	50		
	VMH Lesion (A_1)	0.0094	1	0.0094	6.47*
	$ZnSO_4$ vs. Bulbectomy (B_1)	0.0000	1	0.0000	0.00
	Saline vs. $ZnSO_4$ & Bulb. (B_2)	0.0006	1	0.0006	0.39
Quadratic	$A_1 \times B_1$	0.0035	1	0.0035	2.41
	$A_1 \times B_2$	0.0001	1	0.0001	0.06
	Error	0.0657	45	0.0015	
	Corrected Total	0.0826	50		

*$p < 0.01$

averaged over 18 days. The significant quadratic coefficient indicates that there is a difference between these two groups in the curvature of the regression lines describing the trend.

A significant linear coefficient between bulbectomized and $ZnSO_4$ treated rats was also found in the food intake analysis. This demonstrates a greater increase in food intake over the 18 days for the bulbectomized rats than for the $ZnSO_4$ treated rats.

High-Fat Study

All groups received free access to the high-fat diet for 15 days during which body weight and food intake were measured every third day (Appendix, Tables C and D). The nonlesioned rats had stabilized after 15 days, gaining less than 10 grams every three days and were thus eliminated from the study. The VMH lesioned rats were continued for a total of 60 days to measure their continual increase in body weight and food intake. Analysis of the intercept, linear, and quadratic coefficients for both body weight and food intake of VMH lesioned rats demonstrated no significant differences between groups (Tables 9 and 10).

Histology

Stereotaxic atlases by de Groot (1959) and König and Klippel (1963) were used to evaluate the location and extent of lesions. The lesioned rats' brains, sectioned at 150 μ, sustained extensive semispherical bilateral lesions to the ventromedial hypothalamic area. The de Groot (1959) coordinates between which the lesions extended were: AP = 4.4 to 6.0; H = -2.5 to -4.0; L = 0.0 to 1.5.

Table 9

Analysis of the Intercept, Linear, and Quadratic Coefficients for VMH Lesioned Rats' Body Weight During the Extended High-Fat Diet Test

	Source	SS	df	MS	F
	Groups	3685.81	2	1842.90	0.60
Intercept	Error	58825.85	19	3096.10	
	Corrected Total	62511.66	21		
	Groups	1.120	2	.560	1.40
Linear	Error	7.626	19	.401	
	Corrected Total	8.746	21		
	Groups	0.001	2	.0005	2.5
Quadratic	Error	0.003	19	.0002	
	Corrected Total	0.004	21		

Table 10

Analysis of the Intercept, Linear, and Quadratic Coefficients for VMH Lesioned Rats' Food Intake During the Extended High-Fat Diet Test

	Source	SS	df	MS	F
	Groups	172.32	2	86.16	0.90
Intercept	Error	1823.26	19	95.96	
	Corrected Total	1995.58	21		
	Groups	0.2494	2	0.1247	0.56
Linear	Error	4.1941	19	0.2207	
	Corrected Total	4.4435	21		
	Groups	0.0365	2	0.0182	0.99
Quadratic	Error	0.3510	19	0.0185	
	Corrected Total	0.3875	21		

Those rats which were eliminated from the study during histological examination were generally found to have only unilateral tissue destruction.

Examination of the olfactory bulbs indicated that the damage was localized to the anterior aspects of the olfactory bulbs. No damage to the cortex was found in any of the rats and the neural connections between the cribriform plate and olfactory bulbs were completely severed.

DISCUSSION

The results of the present study indicate that both food intake and body weight in VMH lesioned rats are influenced by diet palatability. In the 24-hour high-fat palatability test it was confirmed that when VMH lesioned rats are allowed free access to a highly palatable diet, they will consume significantly more food and gain more weight than nonlesioned rats. Prolonged access to the high-fat diet also produced significant differences between VMH lesioned and nonlesioned rats.

Responses to unpalatable diets were not as definite as responses to palatable diets. The 24-hour quinine adulterated diet palatability test did not produce any differences in body weight between VMH lesioned and nonlesioned rats. This finding is consistent with several studies which have demonstrated no difference in body weight between VMH lesioned and nonlesioned rats when they are maintained on an unpalatable diet (Ferguson & Keesey, 1975; Franklin & Herberg, 1974; Sclafani & Kluge, 1974; Sclafani, Springer, & Kluge, 1976). There are, however, several possible alternative explanations for these findings. The novelty of the quinine adulterated diet and its availability for only 24 hours might be too brief to allow any between groups differences. Also, the amount of spillage with the quinine-adulterated diet, which made analysis of food intake impossible, could have obscured any differences between VMH lesioned and nonlesioned groups.

Potential difficulties associated with short term testing with unpalatable foods were surmounted by long term measurement of food intake and body weight with the butyric acid-adulterated diet. Significant differences in body weight and food intake were obtained between VMH lesioned and nonlesioned rats. This finding supports earlier interpretations of the VMH syndrome, that VMH lesioned rats are finicky and thus consume less of an unpalatable diet (Corbit & Stellar, 1964; Miller, Bailey, & Stevenson, 1950; Panksepp, 1971). However, recent studies where VMH lesioned rats were maintained at their preoperative weight levels have not found differences in body weight or food intake with unpalatable foods (Ferguson & Keesey, 1975; Franklin & Herberg, 1974; Sclafani & Kluge, 1974; Sclafani, et al., 1976).

The present results are dissimilar to the findings of the preceding studies: VMH lesioned rats lost significantly more weight than nonlesioned rats and the amount of food intake was also significantly lower for the VMH lesioned rats. It's possible, however, that the butyric acid-adulterated diet is more unpalatable than the quinine diet used in those studies reporting no differences.

A further possible explanation for the above finding might be that the lesions did destroy tissue in the subfornical area lateral to the ventromedial nucleus. Several studies (Bevan, 1973; Graff & Stellar, 1962; Gunion, Wellman, Luttmers, & Peters, 1977; Sclafani, et al., 1976) have proposed that damage in this area increases the responsiveness of rats to diet palatability. They have found that

the more lateral the VMH lesion extends, the more finicky the rat. This suggests that obesity and finickiness might be controlled by separate neural mechanisms.

According to the results of the present study, the impact of the olfactory system on food intake and body weight in VMH lesioned rats is ambiguous. The present experiment found no significant differences in body weight with palatable diets between rats with olfactory deficits and those with unimpaired olfactory sensory capabilities. This finding differs from that of Larue and Le Magnen (1970), who found larger weight gains in bulbectomized VMH lesioned rats than in nonbulbectomized VMH lesioned rats. One possible explanation for this difference in body weights could be the difference in the amount of olfactory bulb tissue destroyed. Although Larue and Le Magnen (1970) did not report the extent of damage to the olfactory bulbs, it is noted in other studies that use the bulbectomy technique that the amount of tissue destroyed is quite extensive (Cain, 1974; Cain & Paxinos, 1974; Larue & Le Magnen, 1972). The histological examination of olfactory damage in the present study demonstrated complete separation of the olfactory bulbs from peripheral connections but less total tissue damage to the bulbs than is typically reported.

A second interpretation of the differences between the present experiment and that of Larue and Le Magnen (1970) is the effectiveness of the VMH lesions. In two separate experiments they obtained final mean body weights, for VMH lesioned rats, of 393.7 grams and

460.3 grams. Following bulbectomy these mean weights increased to 531.6 grams and 533.5 grams, respectively, indicating an additive effect of the bulbectomy. The present experiment obtained final mean body weights of 530.5 grams for the VMH-S group and 500.4 grams for the VMH-B group. It thus appears that the VMH lesions, in the present study, were more effective in producing larger increases in body weight. Therefore, lesions which produce large increases in weight gain might obscure the effects of bulbectomy.

A difference in body weight between rats with bulbectomies and those treated with saline was found on the 24-hour quinine adulterated-diet palatability test. The rats with the bulbectomy treatment lost significantly more weight than saline treated rats. This effect is difficult to explain in view of the fact that there were no differences between these groups on any other test. It would also be anticipated that rats with olfactory deficits would not be as sensitive to unpalatable diets and thus would gain more weight rather than lose weight.

The $ZnSO_4$ and bulbectomy techniques used to produce olfactory deficits induced similar behavioral effects. A difference was noted between these two groups in food intake during the butyric acid-adulterated diet study. However, these results must be qualified because the analysis considers all 18 days of data and regeneration of peripheral olfactory sensory tissue in the $ZnSO_4$ rats should have started. Therefore, this comparison is not actually between two equally olfactory impaired groups. This

regeneration was substantiated by the third olfactory discrimination test of VMH lesioned rats. All VMH-Zn treated rats responded identically to the VMH-S group, whereas the VMH-B rats were still deficient in their olfactory discrimination capabilities. This demonstrates the importance of using olfactory discrimination test, such as the one used in this experiment, to demonstrate the effectiveness of treatments which presumably impair olfactory function.

In summary olfactory impairment induced by two different treatments did not influence the feeding behavior of VMH lesioned rats. Although these results are opposite those of Larue and Le Magnen (1970), they do support the recent cephalic phase hypothesis developed by Powley (1977). This hypothesis states that VMH lesions produce an exaggeration of cephalic reflexes, which are "adjustments in energy metabolism made on contact with food or food cues" (Powley, 1977, P. 99). The elimination of sensory cues, e.g. olfaction, should not produce an increase in food intake or weight gain according to this theory. The present data in contrast to those of Larue and Le Magnen (1970) are consistent with this interpretation.

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APPENDIX

Table A

Mean Body Weight (Grams) During Butyric Acid-Adulterated Diet Testing

	Group					
Days	C-S	C-Zn	C-B	VMH-S	VMH-Zn	VMH-B
0	250.3	251.4	251.5	250.0	254.2	258.7
1	247.3	249.0	248.4	248.3	252.6	255.3
2	245.1	246.1	245.1	244.7	249.3	253.9
3	245.4	245.8	242.7	244.3	246.3	251.4
4	245.7	244.8	242.6	240.3	247.0	250.3
5	245.3	244.3	242.3	239.3	245.3	249.4
6	244.8	244.9	242.2	238.5	244.0	247.1
7	243.8	243.9	240.9	237.3	242.6	246.1
8	243.9	242.8	240.5	236.3	241.4	246.4
9	244.0	243.5	241.1	236.5	240.9	244.7
10	243.6	243.0	240.9	235.0	240.0	243.6
11	245.4	243.6	242.2	234.3	239.3	242.9
12	244.9	245.1	242.6	234.0	239.0	243.7
13	243.7	245.9	244.8	235.8	239.0	243.9
14	244.6	246.3	245.0	235.8	237.9	244.1
15	243.9	244.5	243.2	233.8	236.8	242.4
16	243.4	245.1	244.3	233.5	235.6	242.0
17	244.6	246.5	243.8	232.5	235.8	243.6
18	244.2	246.3	245.1	231.7	235.1	244.3

Table B

Mean Food Intake (Grams) During Butyric Acid-Adulterated Diet Testing

	Group					
Days	C-S	C-Zn	C-B	VMH-S	VMH-Zn	VMH-B
1	15.3	14.4	12.8	11.7	11.0	10.7
2	14.7	13.9	13.8	9.8	11.7	12.3
3	21.3	20.0	16.8	15.0	14.7	15.0
4	23.7	21.1	20.1	14.0	16.0	17.6
5	25.0	23.4	22.8	15.7	16.2	17.4
6	24.1	23.5	23.0	16.8	15.9	18.6
7	24.9	23.5	25.2	18.3	16.9	18.4
8	25.0	24.6	24.7	17.5	17.1	16.6
9	26.7	25.3	26.5	21.3	18.0	18.9
10	28.0	25.8	30.2	18.7	21.3	21.4
11	27.9	25.5	27.3	18.8	20.3	21.3
12	29.2	30.1	20.8	22.3	21.7	22.7
13	26.8	27.6	30.7	23.0	20.7	25.7
14	29.1	28.0	30.5	23.2	20.0	24.7
15	26.0	27.4	27.9	21.8	18.7	25.1
16	26.7	26.3	28.0	21.5	19.7	24.7
17	27.6	30.3	28.7	21.3	21.0	26.4
18	27.4	28.6	31.3	22.8	21.3	27.3

Table C

Mean Body Weight (Grams) During Extended

High-Fat Palatability Testing

	Group					
Days	C-S	C-Zn	C-B	VMH-S	VMH-Zn	VMH-B
0	244.2	246.3	245.1	231.7	235.1	244.3
3	269.6	270.5	275.1	267.7	270.7	279.7
6	281.1	282.9	285.5	297.8	302.4	305.9
9	291.6	292.8	293.7	326.7	337.9	330.7
12	295.9	296.3	299.3	354.8	367.7	357.1
15	298.6	302.0	301.0	379.0	393.6	379.0
18	-----	-----	-----	405.7	417.4	398.3
21	-----	-----	-----	422.0	433.0	414.3
24	-----	-----	-----	438.0	455.7	429.9
27	-----	-----	-----	452.8	472.7	440.1
30	-----	-----	-----	465.3	485.3	444.3
33	-----	-----	-----	477.5	501.4	455.9
36	-----	-----	-----	490.3	508.9	467.9
39	-----	-----	-----	498.3	514.6	470.4
42	-----	-----	-----	506.3	526.9	477.1
45	-----	-----	-----	507.2	535.2	479.0
48	-----	-----	-----	515.8	538.0	488.4
51	-----	-----	-----	524.2	542.2	494.0
54	-----	-----	-----	524.5	543.1	495.6
57	-----	-----	-----	530.0	545.3	501.9
60	-----	-----	-----	530.5	542.7	500.4

Table D

Mean Three-Day Food Intake (Grams) During

Extended High-Fat Diet Testing

	Group					
Days	C-S	C-Zn	C-B	VMH-S	VMH-Zn	VMH-B
3	53.3	53.6	57.8	60.9	61.6	62.7
6	46.7	47.5	49.3	76.7	74.4	71.9
9	44.0	41.8	43.8	84.5	93.3	80.9
12	38.3	38.0	37.9	84.3	90.1	75.4
15	35.8	38.8	35.2	84.5	88.2	70.6
18	----	----	----	81.2	81.3	70.9
21	----	----	----	72.0	70.7	63.7
24	----	----	----	75.5	73.0	62.4
27	----	----	----	63.7	67.0	58.3
30	----	----	----	56.8	58.9	44.6
33	----	----	----	57.7	62.0	52.4
36	----	----	----	51.8	51.4	51.7
39	----	----	----	51.0	50.1	42.6
42	----	----	----	47.7	47.8	44.0
45	----	----	----	47.2	47.7	43.3
48	----	----	----	45.0	44.9	41.9
51	----	----	----	44.8	40.0	41.0
54	----	----	----	39.0	42.4	37.3
57	----	----	----	38.0	35.1	38.6
60	----	----	----	34.5	33.6	33.3