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Central nervous system control of food intake and diurnal rhythms.

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CENTRAL NERVOUS SYSTEM CONTROL

OF

FOOD INTAKE AND DIURNAL RHYTHMS

A Dissertation Presented

By

RICARDO ENG

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ABSTRACT

CENTRAL NERVOUS SYSTEM CONTROL

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FOOD INTAKE AND DIURNAL RHYTHMS

September 1979

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Discrete anodal electrolytic lesions of the paraventricular nucleus produce hyperphagia and obesity in rats. The lesions did not affect water/food ratio , normal estrous cycling, or reactivity to an air puff. These measures were comparable to that of controls. However, the lesioned rats grew longer linearly than control rats. Asymmetrical lesions consisting of a paraventricular nucleus lesion paired with a contralateral dorsolateral tegmental lesion also produced hyperphagia and obesity with no disruption of the other measures mentioned above. This suggests that longitudinal pathways are involved in the regulation of food intake. The paraventricular nucleus appears to be the most rostral site of a neural circuit mediating food intake with pathways coursing through the midbrain.

Rats with paraventricular nucleus lesions had normal diurnal and circadian rhythms of feeding and activity. Bilateral parasagittal knife cuts severing the lateral connections of the suprachiasmatic

nucleus reduced activity but had no effect on body weight or diurnal rhythms of eating, drinking, and activity. More caudally placed parasagittal cuts produced hyperphagia, obesity, and hypoactivity. These latter cuts abolished feeding rhythms but not activity rhythms. Surgical isolation of the suprachiasmatic nucleus produced a rat with primarily diurnal eating and activity patterns. Horizontal knife cuts below the paraventricular nucleus temporarily disrupted activity rhythms and eating rhythms, but the rats soon recovered. These findings suggest that the pathways mediating rhythms are separable from those mediating food intake.

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INTRODUCTION

Eating or not eating is the final behavioral consequence following the integration of internal and external signals by cells both in the brain and the periphery. These cells integrate signals from various body sites (e.g., brain, liver, gut, adipose tissue, blood), reporting the energy state of the organism and, in turn, issue signals which ultimately (perhaps involving interneurons) direct the motor sequences initiating, maintaining, or terminating eating. The exact manner in which neurons, and which neurons, bring about this behavior is not known. The integrity of the basal hypothalamus seems essential for the regulation of eating but we have only begun to specify more precisely the neurocircuitry involved and how it works.

Large ventromedial hypothalamic lesions disrupt both regulatory and rhythmic components of food intake. In the rat, these lesions produce hyperphagia and obesity, and also disrupt the normal daytime suppression of eating. However, the day/night cyclicity of eating (i.e. the percent eaten in the day vs. the night) may not be controlled by the same neural substrates that control regulatory (i.e. the total amount eaten). Instead, the simultaneous expression of hyperphagia and loss of rhythms may reflect coincidental damage to two adjacent regulatory systems by the large electrolytic lesions typically used in such studies. It is proposed here that regulatory eating and diurnal eating rhythms are mediated by separate neural systems. If this is

the case, then one would expect that somewhere there is a site where a discrete brain lesion would produce hyperphagia and obesity without disrupting diurnal eating rhythms, and vice versa. It is further proposed that the paraventricular nucleus (PVN) is part of a system mediating regulatory eating, whereas the suprachiasmatic nucleus (SCN) is part of a system mediating eating rhythms, the pathways for which remain to be determined. Supporting evidence for this thesis will be reviewed in the following pages. Chapter I will deal with regulatory eating and Chapter II will deal with eating rhythms.

CHAPTER I

CNS CONTROL OF EATING

Hypothalamic Hyperphagia and Obesity

Historical overview. At the beginning of this century the function of the hypothalamus was unknown. Clinical observations (e.g., Frohlich, 1901) described an "adipogenital" syndrome (obesity with failure to mature sexually) associated in some cases with polyuria (diabetes insipidus). Frohlich (1901) attributed the obesity to a tumor of the pituitary. Experimental hypophysectomy in dogs (Crowe, Cushing, and Homans, 1910) seemed to support Frohlich's hypothesis. There were objections (Aschner, 1912) that the obesity might result from damage to the base of the brain as a consequence of the transtemporal approach used in the hypophysectomy. When hypophysectomy was performed via the roof of the mouth, thus avoiding damage to the base of the brain, there was no obesity. Despite these objections, Cushing's view on the role of the pituitary went unchallenged until two of his students (Bailey and Bremer, 1921) showed that puncture of the post-infundibular region of the hypothalamus without damage to the pituitary produced the adipogenital syndrome and diabetes insipidus. This was the beginning of the pituitary-hypothalamic controversy. Smith (1930) using chromic acid lesions, and Keller et al. (1930) using surgical lesions of the hypothalamus also reported the obesity. However, the controversy was not settled until Hetherington and

Ranson (1939; 1940; Hetherington, 1943) conclusively demonstrated that bilateral damage in the ventromedial region of the hypothalamus, not the pituitary, was responsible for the hyperphagia and obesity. The critical observation involved 2-stage surgery. Hypophysectomy alone failed to produce obesity, but subsequent hypothalamic lesions did. The prior removal of the pituitary ruled out any possible involvement of the pituitary in the etiology of the obesity.

The dual center hypothesis. The ventromedial (Brobeck et al. 1943), and lateral (Anand and Brobeck, 1951a) hypothalamic areas were proposed as sites for the control of food intake. The "dual center" hypothesis of Anand and Brobeck (1951b) proposed that the ventromedial hypothalamus (VMH) was a "satiety center" which suppressed the "eating center" located in the lateral hypothalamus (LH). The dual center hypothesis generated and guided considerable research on the central nervous system control of eating (for reviews see Stevenson, 1969; Grossman, 1975, 1979; Lytle, 1978). However, the seductive simplicity of this hypothesis led investigators to the mistaken belief that they had identified the "centers" controlling eating and satiety by naming them, and that all that remained was to examine the factors necessary for the development of hyperphagia and obesity. Certainly, the large electrolytic lesions of the VMH and LH provided supportive evidence at that time (Anand and Brobeck, 1951a; Teitelbaum and Epstein, 1962).

Longitudinal systems controlling eating. In the past, the dual center hypothesis focused on the ventromedial nucleus and the region just lateral to it, detracting attention from more anterior or caudal brain sites. More recently, due to the advent of more selective lesioning methods such as knife cuts (which damage fibers crossing the plane of the knife, but minimize neuronal damage, e.g., Gold, 1970; Gold et al., 1977; Grossman and Grossman, 1977) transmitter specific neurotoxins (e.g., Ahlskog and Hoebel, 1973; Saller and Stricker, 1976; Breisch et al., 1976) and selective stimulation via intracranial microinfusions of transmitters (e.g., Leibowitz, 1978a) researchers have re-discovered that eating regulation must involve more than isolated brain centers.

The original term ventromedial hypothalamic area was shortened to ventromedial hypothalamus (VMH) and then was confused with the ventromedial nucleus (VMN). Thus the VMN was erroneously implicated in the control of food intake. This confusion was clarified by Gold (1973) who showed, by using discrete electrolytic lesions, that the critical area damage which produced hyperphagia and obesity was not the VMN, but rather lay immediately rostral to it. This finding has since been replicated (Coscina et al., 1976). Hyperphagia and obesity can also be produced by parasagittal knife cuts (Albert and Storlien, 1969; Sclafani and Grossman, 1969; Gold, 1970; Sclafani, 1971; Paxinos and Bindra, 1973). The most effective placement for these parasagittal cuts is rostral to the VMN and LH and coincides with

Gold's (1973) electrolytic lesion findings. Coronal knife cuts rostral to the anterior tip of the VMN also produce hyperphagia and obesity (Grossman, 1971; Paxinos and Bindra, 1972; Storlien and Albert, 1972). The above effects could be due to interruption of caudal connections of the rostral hypothalamus to the posterior hypothalamus and/or brainstem. If so, this would be evidence for longitudinal systems involved in food intake. In support of this, a parasagittal knife-cut rostrolateral to the VMN combined with a contralateral mammillary area lesion, an approach designed to asymmetrically damage the same system bilaterally, produces hyperphagia and obesity (Gold et al., 1972). A finer analysis of the caudal connections mediating food intake (Grossman and Hennessy, 1976; Hennessy and Grossman, 1976; McDermott et al., 1977) implicates the medial components of the medial forebrain bundle (MFB) as it courses through the perifornical posterior hypothalamus. In cats, bilateral electrolytic lesions of the dorsolateral tegmentum (Skultety and Gary, 1962; Skultety, 1966) produce hyperphagia and obesity. In rats, tegmental coronal knife-cuts (Grossman and Grossman, 1977) also produce hyperphagia and obesity. Finally, Gold et al.'s (1977) asymmetrical knife-cuts also support the existence of a longitudinal system running from the area of the PVN through the posterior hypothalamus (possibly via the MFB) into the tegmental area. The posited system appears to become more diffuse as it courses caudally since larger knife-cuts (Gold et al., 1977) or large lesions (Skultety and Gary, 1962) are needed to produce the effect at tegmental levels.

Studies using transmitter-specific neurotoxins also support the concept of longitudinal systems mediating food intake. Microinjections of 6-hydroxydopamine (6-OHDA), which specifically depletes catecholamines, into the ventral tegmentum, where the cell bodies of ascending noradrenergic systems (e.g., the ventral noradrenergic bundle) are located, cause hyperphagia and obesity (Ahlskog and Hoebel, 1973). However, the effect of 6-OHDA lesions appears to be dissociable from that of VMH electrolytic lesions (Ahlskog et al., 1975). Indeed, norepinephrine depletion is not essential for the hyperphagia seen after coronal tegmental knife-cuts (Grossman and Grossman, 1977; Grossman et al., 1977). In some cases 6-OHDA injected into the tegmentum has no effect, whereas small electrolytic lesions in the same area produce a small, diet-dependent hyperphagia and obesity (Lorden et al., 1976). Norepinephrine depletions in the forebrain do not necessarily produce hyperphagia and obesity (Coscina et al., 1973). Thus, the posited damage to the ventral noradrenergic bundle (Gold, 1973; Kapatos and Gold, 1973) may be neither necessary nor sufficient for the expression of VMH hyperphagia and obesity.

Serotonin (5-HT) depletions have been reported to produce hyperphagia and increased body weight (Breisch et al., 1976; Saller and Stricker, 1976). In this respect, the coronal tegmental knife-cuts which produce hyperphagia and obesity significantly deplete forebrain 5-HT (Grossman et al., 1977). However, Saller and Stricker (1976) found that while their rats were hyperphagic and gained weight, they did not become obese. Saller and Stricker attributed these effects

to excess growth hormone, because their rats grew longer in length. Thus the role of 5-HT in hypothalamic obesity is not clear. Furthermore, 5-HT depletions via lesions of the midbrain raphe nuclei (Lorens et al., 1971; Coscina et al., 1972; 1976; Coscina and Stancer, 1977) do not produce hyperphagia and obesity. On the contrary, raphe lesions markedly attenuate or abolish VMH hyperphagia and obesity (Coscina and Stancer, 1977). These conflicting reports may be due to the degree of 5-HT depletion, or to damage to more than 5-HT neurons.

The PVN and food intake. In contrast to Hetherington and Ranson (1940), who identified the ventromedial hypothalamus as the critical hypothalamic site, other early investigators had suggested a role for the anterior hypothalamus in the inhibition of food intake. Camus and Roussy (1922) attributed the obesity to lesions of the arcuate nucleus and the PVN. Biggart and Alexander (1939) emphasized the importance of the anterior hypothalamus. Their lesions damaged ventral and caudal portions of the supraoptic nucleus and the anterior hypothalamus anterior to the median eminence. They believed that such lesions interrupted fibers from the PVN to the hypophysis, and recalled Greving's (1928) suggestion that the PVN controlled fat metabolism. Anterior hypothalamic lesions including damage to the PVN produced hypoglycemia in cats (Barris and Ingram, 1936) and increased insulin sensitivity (Ingram and Barris, 1936). Unilateral PVN stimulation produced hyperglycemia (Lewy and Gassmann, 1935). From clinical observations of brain tumors, the PVN and its tegmental connec-

tions to the brainstem dorsal motor nucleus of the vagus were proposed as an insulin regulating system (Vonderahe, 1937).

The PVN itself has been little explored because early studies (e.g., Hetherington and Ranson, 1940, 1942) concentrated on more caudal hypothalamic sites (ventromedial, dorsomedial, and lateral). Accordingly, there is little direct evidence specifically implicating the PVN in the control of food intake: An early study (Heinbecker et al., 1944) reported hyperphagia and obesity in dogs after hypothalamic puncture lesions which damaged the PVN and/or its efferents. As mentioned earlier, large electrolytic lesions of the anterior hypothalamus which included the PVN, lowered blood sugar levels (Barris and Ingram, 1936), and also lowered growth hormone releasing factor (Sawano et al., 1968). However, the effect of the lesions on food intake and body weight was not tested in either study since the cats were on a restricted feeding schedule.

The early studies suggesting a role for the PVN in the control of food intake were overlooked or bypassed because, for the most part, they emphasized connections to the pituitary at a time when it was becoming apparent that the pituitary was not involved. However the PVN hypothesis once again becomes justifiable in light of several recent findings. One line of evidence comes from the recently demonstrated extrahypothalamic luteinizing hormone releasing hormone (LH-RH) pathways (Knigge et al., 1978), and extrahypothalamic neurophysin pathways and other direct autonomic pathways from the PVN to brainstem nuclei (Saper et al., 1976; Hancock, 1976; Swanson, 1977;

Nilaver et al., 1978) and reciprocal connections to the PVN (Ricardo and Koh, 1978). These pathways may be relevant in view of the reports that vagotomy abolishes VMH hyperphagia and obesity (Powley and Opsahl, 1974), knife-cut-induced obesity (Sawchenko and Gold, 1977) and PVN norepinephrine-induced eating (Leibowitz, unpublished; Sawchenko, 1978). Electrolytic lesions (Gold, 1973) and asymmetrical knife-cuts (Gold et al., 1977) implicate the PVN area as the most rostral terminus of a satiety neurocircuit. Finally norepinephrine microinjection studies (Leibowitz, 1978a) localize the PVN as the most sensitive site for eliciting eating. The discrete electrolytic lesions of the PVN reported in this thesis confirm the role of the nucleus in the control of food intake.

Possible specificity of PVN lesions. It should not be surprising that large lesions of the ventromedial hypothalamus cause a complex syndrome. Besides hyperphagia and obesity, this syndrome includes dietary "finickiness" (Graff and Stellar, 1962), disrupted eating and drinking rhythms (Kakolewski et al., 1971), hypoactivity and disrupted gonadal function (Kennedy and Mitra, 1963), and hyper-reactivity and increased "emotionality" (Grossman, 1966). The complexity of the syndrome reflects both the size of the lesions used and the intricate substrate lesioned. The medial hypothalamus contains aminergic afferents and fibers of passage (Ungerstedt, 1971; Lindvall et al., 1974; Swanson and Hartmann, 1975; Hokfelt et al., 1978), estrogen concentrating cells (Pfaff and Keiner, 1973), adenohypophyseal trophic hor-

mones (Guillemin, 1978), and many newly discovered peptidergic pathways (Buijs et al., 1978; Hokfelt et al., 1978; Knigge et al., 1978), in addition to the traditional neurohypophyseal system (Scharrer and Scharrer, 1954). Thus, it is very probable that discrete PVN lesions may isolate the hyperphagia and obesity from the other components of the VMH syndrome, producing an "elemental" obesity syndrome. However, such a possibility should be tempered by the caveat of our lack of sophistication as to the function(s) of the PVN.

Research strategy. Discrete electrolytic lesions of the PVN should produce hyperphagia and obesity without abolishing the nocturnality of intake, and preserve normal gonadal function. If the PVN is indeed part of longitudinal pathways mediating food intake, then an asymmetrical lesioning approach i.e., a PVN lesion paired with a contralateral dorsolateral tegmentum (DLT) lesion, should produce hyperphagia and obesity comparable to that of bilateral PVN lesions. The effects of PVN lesions on gonadal function and rhythms will be tested via vaginal smears, and by observing nocturnal rhythms of intake. However, since this latter measure comes under "rhythms" it will be dealt with in Chapter II.

Methods

Subjects. Adult female Charles River CD rats (250-300 gm) served as subjects. Rats were housed singly in stainless steel wire-mesh hanging cages, in an air-conditioned room, under cycling lights (12:12

hrs, light/dark). Purina laboratory pellets and tap water was available *ad libitum*. On the occassion of dietary challenges, Purina powdered diet, or a high fat diet (2/3 powder, 1/3 Crisco, by weight) was used.

Surgery. Under Nembutal anesthesia (40 mg/kg, i.p.) rats were placed in a Kopf stereotaxic instrument and received anodal electrolytic lesions (1 mA x 10 sec) bilaterally *via* a non-metal-depositing platinum-iridium (90-10%) electrode (0.3 mm in diameter) insulated with enamel except for 0.5 mm at the tip. The cathode was attached to the ipsilateral ear. The current parameters and mechanisms of lesion formation using this electrode have been previously reported (Gold, 1975). Briefly, oxygen bubbles formed at the anode damage tissue mechanically and the acidity of the hydronium ions (H_3O^+) produced contributes to lesion formation. Stereotaxic coordinates were taken from Konig and Klippel's (1963) atlas and adapted for the adult rats used. With the incisor bar set at - 3.0 mm, the PVN coordinates were: 7.0 mm anterior from ear bar, lateral $\pm$ 0.3 mm from the midline (measured from the sagittal sinus), and 7.7 mm ventral from the dura. For the asymmetrical lesions, a unilateral PVN lesion was placed as above, and paired with a contralateral DLT lesion. The coordinates for the DLT lesion were: 2.3 mm anterior from the ear bar, 1.5 mm lateral, and 6.6 mm ventral from the dura. The lesion parameters were 1 mA x 30 sec. For sham lesions, rats were anesthetized, placed in the stereotaxic and the electrode lowered to within one mm of the

nucleus, but no current was passed.

Measures. Following a one-week settling period after the rats' arrival, bi-weekly body weight and intake data were taken for 14 days pre-operatively and for at least 14 days post-operatively. Post-operative daily vaginal smears were taken over 2 cycles (approximately 8-10 days) to be used as an index of normal ovarian function. Post-operative reactivity to an air puff from an ear syringe delivered to the area around the head, was taken as a measure of emotionality. The emotionality scores to the puff were: 0 (no reaction), 1 (sniffing), 2 (sniffing and moving around), 3 (flinching), 4 (flinching and vocalizing), 5 (jumping off the cage floor, vocalizing). Nose-anal lengths (NAL) were taken at the time of surgery and again just before perfusion. From the NAL and body weight (BW) an obesity index (Lee, 1929) can be calculated using the formula, obesity index = $(Bw^{1/3})/$ (NAL in millimeters) x (10^4). A range from 290-310 is considered normal and 320+ is considered obese.

Histology. At the conclusion of the experiment, rats received an overdose of Nembutal (80 mg/kg, i.p.) and were perfused intracardially with physiological saline followed by 10% formalin. Brains were removed from the skulls using rongeurs, and placed in 10% formalin for 24 hours followed by 24 hours in sucrose-formalin. Frozen coronal, or horizontal sections (for the bilateral PVN, or the PVN x DLT respectively) of 40 microns were cut and every fourth section saved and mounted on glass slides for staining with Cresyl violet. Lesion

reconstructions consisted of camera lucida tracings of sections through the extent of the lesion. Two observers who had no knowledge of the behavioral results rated the extent of damage to the PVN.

Statistical analysis. After the rats were sorted into groups based on the amount of PVN damage, group means for body weight, intake, and obesity index, and their respective standard errors were calculated. These data were then analyzed using two-tailed Student's t tests for between-groups comparisons. In addition, a one-way analysis of variance, followed by a Newman-Keuls or Duncan's multiple range test was done on body weight, and on linear growth.

The extent of damage to the PVN was determined by projecting the brain section (13X magnification) onto a grid pattern (1 square = 0.5 mm). An intact PVN covered 7 squares on the grid. The extent of damage was calculated by dividing the number of squares covered by any remaining PVN by 7. This was converted to a percentage, and subtracted from one hundred. Thus, total damage to the nucleus would give a value of one hundred percent. A linear regression analysis was then done between extent of damage to the nucleus, and the subsequent body weight and food intake changes.

Results

Histology. Seven rats sustained total (100%) damage to the PVN. Six rats sustained partial (50-87%) damage, and seven had no damage to the PVN. Seven rats received a sham operation. Representative lesion

reconstructions are shown in the accompanying figures (Figs. 1-3.).

Intakes and body weights. These data are summarized in Table 1. Pre-operatively, there were no significant differences in food and water intake, water-to-food ratios, or body-weight change between any of the groups. Total PVN destruction led to a significant increase in food ($p < 01$, $t = 9.82$, $df = 12$) and water ($p < .01$, $t = 5.41$, $df = 12$) intakes, and body weight ($p < .01$, $t = 18.6$, $df = 12$) compared to sham operates, or to control lesions ($p < .01$, $t = 8.84, 3.98, 11.6$ respectively, $df = 12$). Rats with total destruction of the PVN were significantly more obese ($p < .01$, $t = 8.2, 4.72$) than either sham operated or lesioned controls. The mean Lee index for the rats with total PVN lesions was 330 ± 2.08, definitely in the obese range in contrast to mean indices of 307 and 299.3 for the control lesioned and sham operated rats, respectively. Rats with partial PVN lesions had a Lee index of 325 ± 4.0. Total PVN lesions did not significantly affect the water-to-food ratios ($p > .10$, $t = .82, .41$, $df = 12$) compared to shams or lesioned controls. Rats with total PVN lesions differed significantly from the partially-lesioned group in post-operative weight increase ($p < .01$, $t = 3.58$, $df = 11$). There was a significant difference in their total post-operative food intake ($p < .01$, $t = 7.15$, $df = 11$). Rats with total PVN lesions increased food intake 70% from pre-operative levels, compared to a 48% increase for the rats with partial PVN damage.

Partial damage (50 - 87%) to the PVN, when compared to shams,

Fig. 1. Representative total PVN lesion. The extent of the lesion is shown by the hatched area. The outline of the PVN is represented by dotted lines.

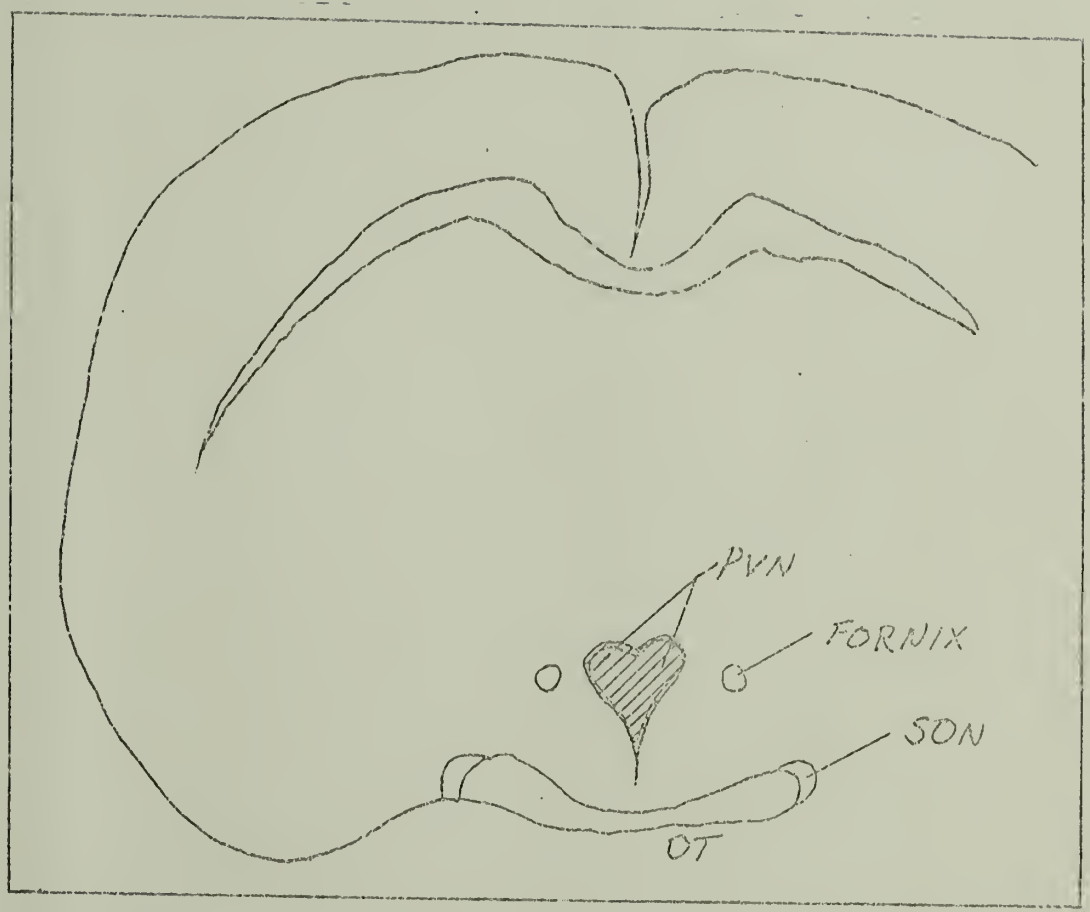
PVN
FORNIX
SON
OT

Fig. 2. Representative partial PVN lesion.

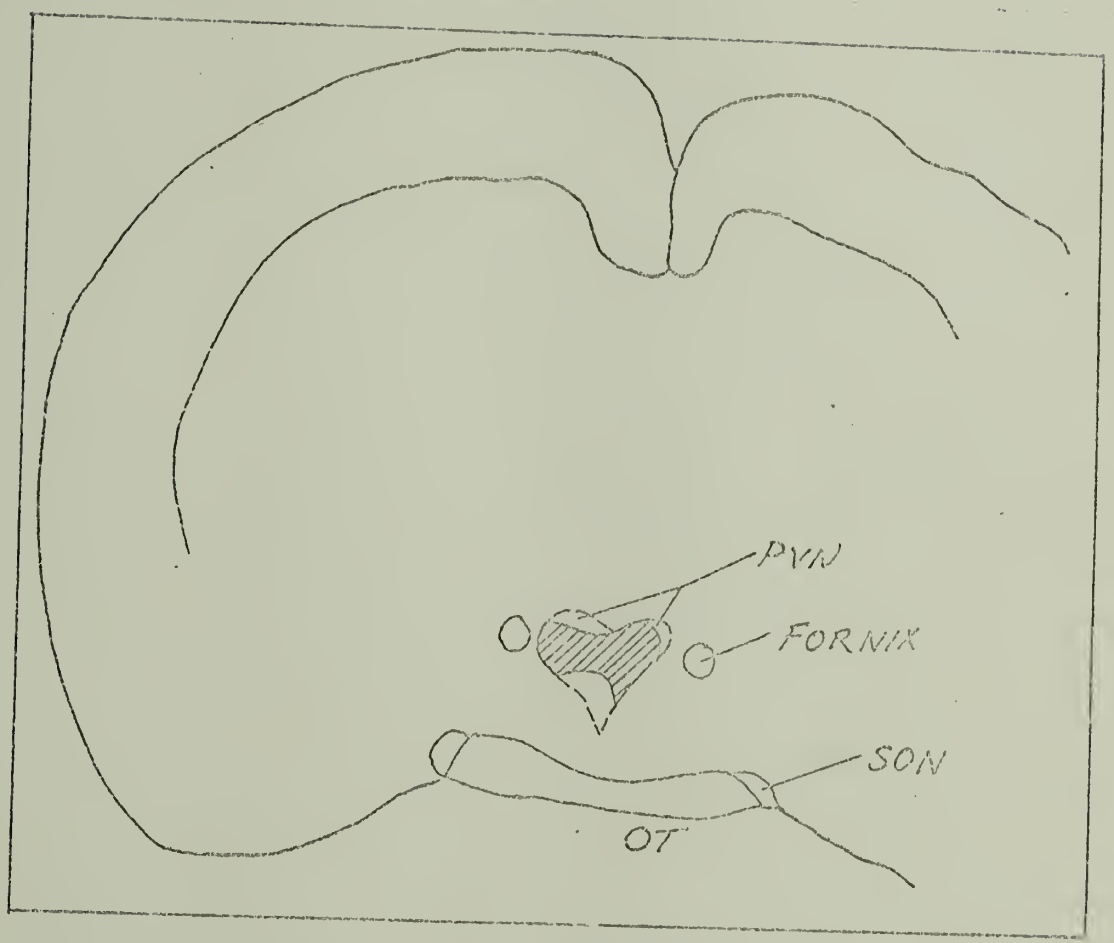
PVN
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Fig. 3. Representative asymmetrical (PVN x DLT) lesion.

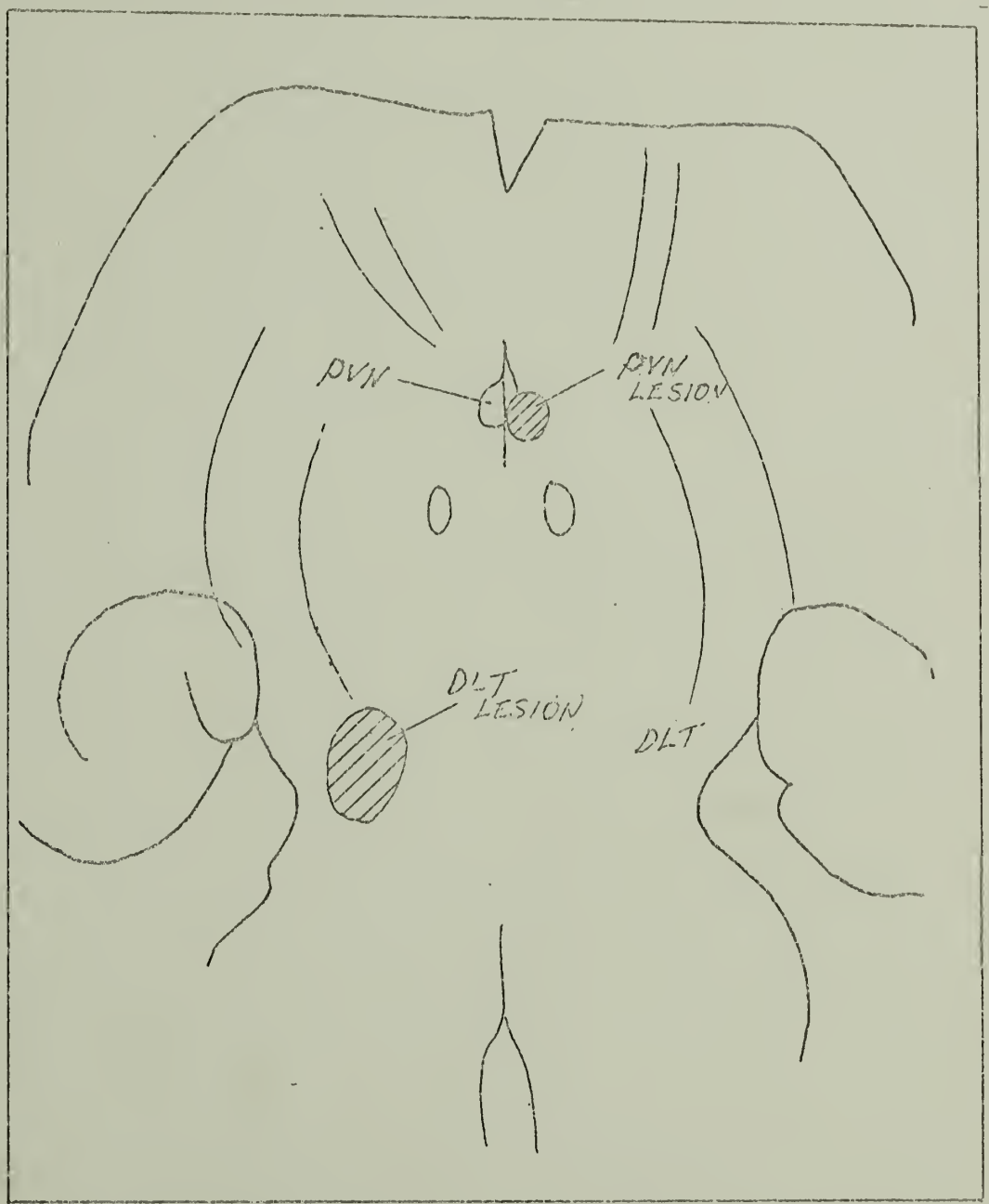

PVN
PVN LESION
DLT LESION
DLT

Table 1

Mean Daily Intakes, Water/Food Ratios (W/F),

and Body Weight Changes: Pre- and Post-Operatively

	Mean Pre-Operative Measures (± S.E.M.)				Mean Post-Operative Measures (±S.E.M.)				
	Food (g/d)	Water (g/d)	W/F	Body Weight Change	Food (g/d)	Water (g/d)	W/F	Body Weight Change	Mean Lee Index
Total PVN Lesioned Rats	23.16 (±0.72)	32.15 (±1.09)	1.39 (±0.056)	0.73 (±.25)	39.56* (±1.13)	52.49* (±3.18)	1.29 (±.078)	6.2 (±.31)	329.98* (±2.08)
Partial PVN Lesioned Rats	22.81 (±1.25)	33.21 (±2.18)	1.46 (±.089)	0.85 (±.28)	33.78* (±2.62)	43.9* (±3.8)	1.33 (±.067)	3.36* (±0.73)	325.27* (±4.0)
Control Lesioned Rats	21.63 (±0.78)	31.19 (±2.78)	1.43 (±.085)	0.82 (±.093)	26.4 (±1.29)	36.7 (±2.36)	1.39 (±.23)	0.88 (±.34)	307.0 (±4.4)
Sham Lesioned Rats	20.51 (±.87)	32.97 (±0.98)	1.61 (±.057)	0.61 (±.14)	22.13 (±1.26)	31.72 (±2.13)	1.39 (±.094)	0.25 (±.082)	299.3 (±3.1)

resulted in significant increases in food and water intakes ($p < .05$, $t = 3.73, 2.79$, $df = 11$), body weight ($p < .01$, $t = 4.23$, $df = 11$) and Lee index ($p < .01$, $t = 5.13$, $df = 11$). Compared to control lesions, partial PVN lesions induced significant increases in food intake ($p < .05$, $t = 2.53$, $df = 11$), body weight ($p < .05$, $t = 3.08$, $df = 11$), and Lee index ($p < .05$, $t = 3.07$, $df = 11$), but the increase in water intake was not significant ($p > .10$, $t = 1.61$, $df = 11$). Rats with partial PVN lesions were obese, with a mean index of 325.27. However, rats with partial PVN lesions did not differ significantly from rats with total PVN lesions in the Lee index ($p > .10$, $t = 1.04$, $df = 11$). It should be noted that the 50% damage to the PVN consisted of a unilateral PVN lesion, the contralateral nucleus being spared. This unilateral lesion did not produce hyperphagia and obesity.

A linear regression analysis of extent of damage to the PVN (Y) vs. body weight gain (X) yielded an $\underline{r}$ of 0.87, and a regression equation of $y = 16.46X + 1.32$. This correlation was highly significant ($p < .01$, critical $\underline{r} = 0.561$, $df = 18$). A regression analysis of postoperative food intake gave similar results, with an $\underline{r} = 0.87$, and a regression equation of $Y = 5.69(X) - 132.56$ ($p < .01$, $df = 18$). The scatter plots and regression lines are shown in Figures 4 and 5.

The results of the analysis of variance of the postoperative body weight gains was highly significant ($p < .01$, $F = 50.62$, $df = 3.23$). The summary of the analysis is presented in Table 2. All groups were significantly different from each other except for the control lesion vs. sham comparison (Newman-Keuls, Table 3; Duncan's Multiple Range,

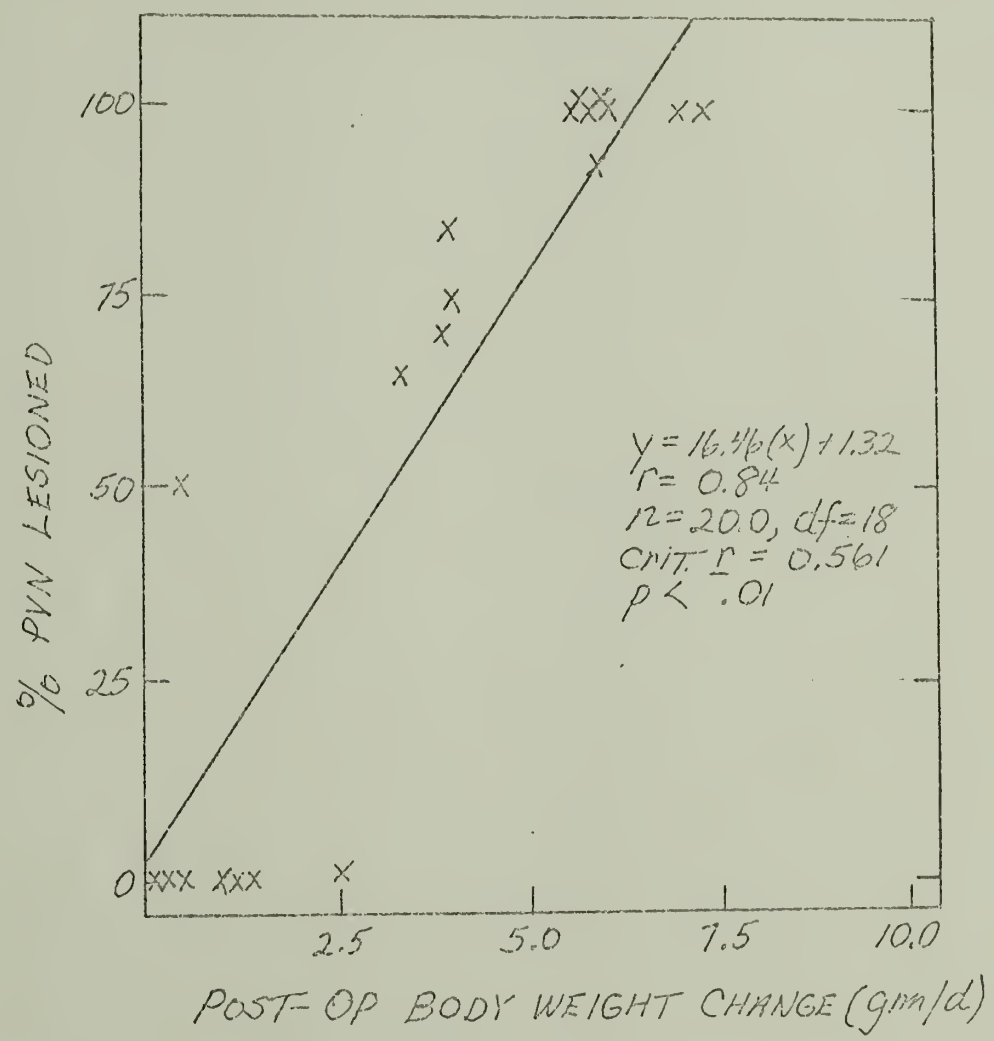


Fig. 4. Scatter plot and linear regression line for % PVN damaged vs. post-operative body weight change.

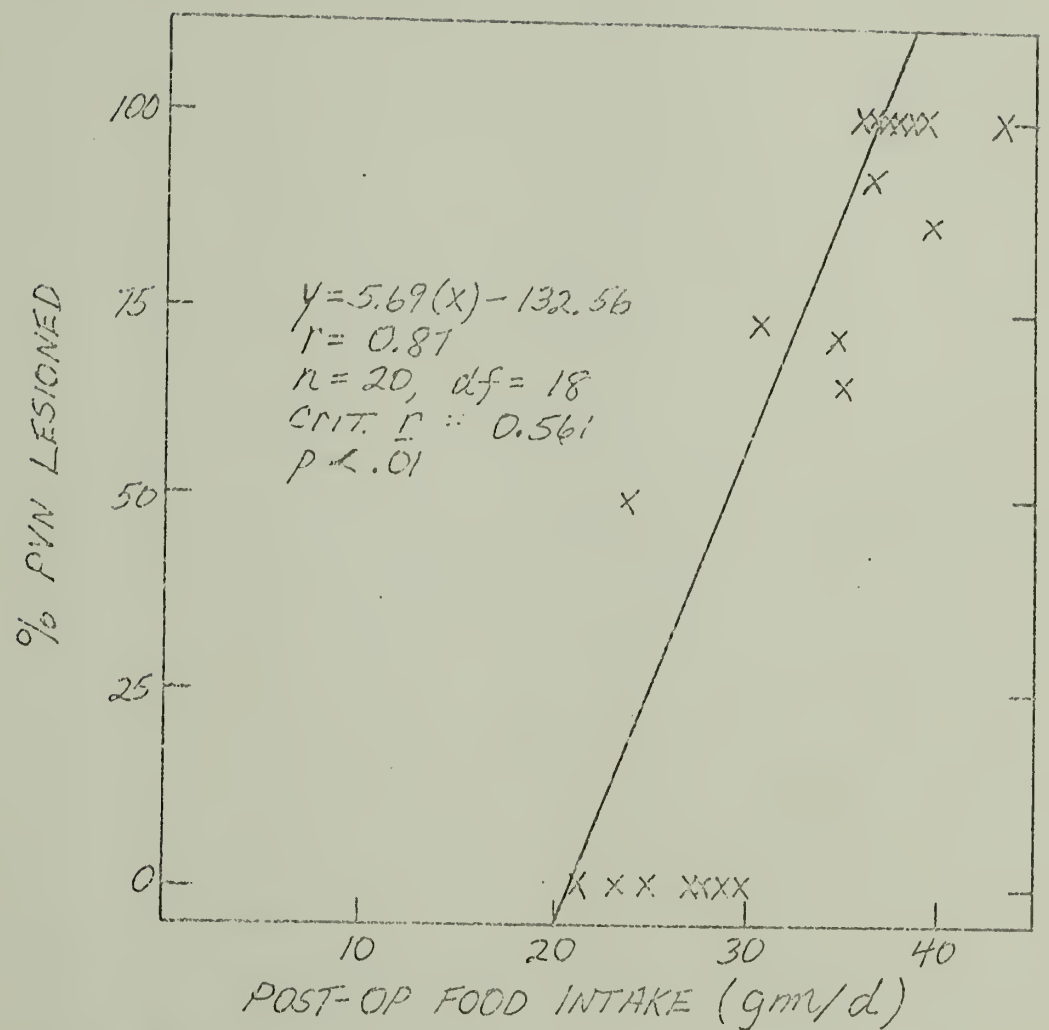


Fig. 5. Scatter plot and linear regression line for % PVN damaged vs. post-operative food intake.

Table 2

Summary of the Analysis of Variance

for Post-Operative Body Weight Changes

	SS	df	MS	F	P
bg	154.89	3	56.63	50.60	.01
wg	23.36	23	1.02		

Mean Post-Operative Body Weight Change (g/d)

Total PVN lesion	6.2	±	.746
Partial PVN lesion	3.52	±	1.77
Control lesion	0.877	±	.835
Sham lesion	.257	±	.203

Table 3

Newman-Keuls Paired Comparisons

for Post-Operative Body Weight Change

	4 Total PVN Lesion	3 Partial PVN Lesion	2 Control Lesion	1 Sham Lesion
4 Total PVN Lesion	-	2.11*	5.37*	5.94*
3 Partial PVN Lesion	-	-	3.26*	3.83*
2 Control Lesion	-	-	-	0.57 N.S.
1 Sham Lesion	-	-	-	-

*$p < .01$

EF .01

2	3	4
3.89	4.45	4.80
.93	1.07	1.15

Table 4

Duncan's Multiple Range Test

for Post-Operative Body Weight Change

	Partial PVN Lesion	Sham Lesion	Control Lesion	Total PVN Lesion
Partial PVN Lesion	-	3.26*	2. 64*	2.60*
Sham Lesion	-	-	0.62	5.9*
Control Lesion	-	-	-	5.32*
Total PVN Lesion	-	-	-	-

*$P < .01$

$R_4 = 1.03$

$R_3 = 1.002$

$R_2 = 0.950$

Ranks

X_4: Sham Lesion

X_3: Control Lesion

X_2: Partial PVN Lesion

X_1: Total PVN Lesion

Table 4).

Estrous cycling. All rats showed the normal 4-5 day cycle as determined by observation of the daily vaginal smears.

Reactivity. There were no differences in reactivity to the air puff among the groups. Typically, rats showed a sniffing and moving-around response, sometimes a flinch, but there were no vocalizations or jumping off the cage floor.

Linear growth. Because of the different post-operative survival times, the nose-anal lengths (NAL) were transformed to daily growth (mm./day). The transformed data were analyzed via analysis of variance and Duncan's multiple range test. The results, summarized in Tables 5 and 6, show that rats with total PVN lesions grew at a significantly faster rate ($p < .01$) than the other groups, which did not significantly differ from each other ($p > .10$).

Asymmetrical lesions. Because of the small number of animals (4) which received a unilateral PVN lesion paired with a contralateral DLT lesion, these data will be presented anecdotally. A typical lesion is shown in Figure 3. Rats with such asymmetrical damage gained 4.0 grams/day, and had a Lee index comparable to that of the rats with partial PVN lesions (329). These asymmetrically lesioned rats were hyperphagic (60% increase over pre-operative intake), but not polydipsic (water-to-food ratios were normal). They had normal estrous cycling,

Table 5. Summary of Anova on linear growth (mm/day).

Table 5

Summary of the Analysis of Variance for Post-Operative Increases in Nose-Anal Length (N.A.L.)

	SS	MS	df	F	P
bg	.06017	.02057	3	3.10	.05
wg	.1496	.008504	23		

Mean Post-Operative Increases in N.A.L. (MM/d) ± S.E.M.

Total PVN lesion	.187 ± .053
Partial PVN lesion	.0694 ± .012
Control lesion	.092 ± .054
Sham lesion	.0789 ± .032

Table 6

Duncan's Multiple Range Test

for Post-Operative Increases in Nose-Anal Length

	Partial PVN Lesion	Sham Lesion	Control Lesion	Total PVN Lesion
Partial PVN Lesion	-	.0095	.0231	.118*
Sham Lesion	-	-	.0136	.1081*
Control Lesion	-	-	-	.0945*
Total PVN Lesion	-	-	-	-

*$p < .01$

CRIT. R.

R_4 = .08235

R_3 = .07986

R_2 = .07585

Ranks

X_2: Partial PVN lesion

X_4: Partial Sham lesion

X_3: Control lesion

X_1: Total PVN lesion

and were also normal in their response to the air puff.

Discussion

The results confirm earlier suggestions that the PVN is involved in the control of food intake and body weight (Heinbecker et al., 1944; Gold et al., 1977; Leibowitz, 1978a). The present results suggest a role for the PVN itself, as damage to the nucleus is highly correlated with hyperphagia, increased body weight, and obesity. There was no polydipsia as defined by water/food ratios. Damage to the PVN did not disturb estrous cycling, confirming an earlier report by Brown-Grant et al., (1977). The normal reactivity after PVN lesions is congruent with the reports after knife cuts (Paxinos and Bindra, 1972; Gold et al., 1977). Taken together these findings support the hypothesis that PVN lesions are more specific than VMH lesions in producing hyperphagia and obesity. The persistence of normal circadian rhythms in behavior following PVN lesions will be assessed in part II.

The results of the asymmetrical lesions (PVN x DLT) suggest that the PVN is part of longitudinal pathways coursing through the tegmentum (Skultety and Gary, 1962; Peters et al., submitted, 1979) and asymmetrical knife cuts (Gold et al., 1977). The asymmetrical lesions did not affect estrous cycling, reactivity to the air puff, or cause polydipsia. In this respect, the asymmetrical lesions appear to be equivalent to bilateral PVN lesions. The lesser magnitude of the body-weight increase can be accounted for in terms of the system's

becoming more diffuse at tegmental levels, thus requiring large lesions (Skultety and Gary, 1962) or knife cuts (Gold et al., 1977) to fully interrupt it.

The identity of transmitter system(s) involved in this longitudinal system remains a mystery. In view of the rich aminergic innervation of the medial hypothalamus (Hokfelt et al., 1978), especially the catecholaminergic input to the PVN (Lindvall and Bjorklund, 1974), an aminergic system is a good candidate. This would be consistent with the fact that Ahlskog and Hoebel (1973) produced hyperphagia after injections of the catecholamine-specific neurotoxin 6-OHDA into the tegmentum, and norepinephrine microinjections are most effective in eliciting eating when placed in the PVN (Leibowitz, 1978a).

The relationship between the system identified by Leibowitz (1978a) and the system revealed by knife cuts and PVN lesions is not clear. It could be that norepinephrine stimulates food intake via disinhibition, i.e., by inhibition of satiety. Such an interpretation would be internally consistent with the alpha-eating and beta-satiety adrenergic "dual center" model (Leibowitz, 1970). In support of such a possibility, iontophoretic application of norepinephrine has been shown to inhibit firing of PVN neurons (Moss et al., 1972). Furthermore, sub-(eating)threshold doses of norepinephrine (0.015-0.37 nmoles) triple the size of the meal intake, a fact which would be consistent with an inhibition of satiety (Ritter and Epstein, 1975). However, norepinephrine levels are inversely correlated with weight-gains after radio-frequency lesions of the medial hypothalamus (Coscina et al.,

1976). Such a relationship would not be expected if norepinephrine is suppressing a PVN satiety system. If this were the case, depletions of norepinephrine should lead to increased satiety rather than increased eating. Similarly, if norepinephrine is suppressing satiety, then the hyperphagia and obesity seen after PVN knife cuts (Gold et al., 1977) can not be attributed to damage to noradrenergic inputs to the PVN, because again the expected result would be increased satiety and body weight loss. There is a report of hypophagia after transections of the tractus filiformis, a major noradrenergic input to the PVN (O'Donohue et al., 1978). However, the effect is transient and the animals recover despite nearly a 60% depletion of noradrenergic input to the nucleus.

An alternative interpretation is that the pathways mediating noradrenergic eating and knife-cut hyperphagia are different. Noradrenergic eating responses remain intact after knife cuts beside the PVN (Aravich et al., 1978), which result in hyperphagia and obesity. Thus, it is very likely that knife cuts and lesions produce hyperphagia and obesity via damage to PVN connections which are not adrenergic and which may be efferent.

Other evidence argues against an exclusive catecholaminergic mediation of VMH hyperphagia. The 6-OHDA effect is additive to VMH lesions (Ahlskog et al., 1975). Furthermore selective depletion of PVN norepinephrine (56% decrease) by discrete knife cuts along the PVN has no permanent effects on food intake (O'Donohue et al., 1978). Serotonin probably is also involved because depletions of this indolea-

mine are correlated with the hyperphagia produced by tegmental knife cuts (Grossman et al., 1977), and VMH lesions (Coscina et al., 1976). However, hyperphagia after serotonin depletions does not produce obesity (Saller and Stricker, 1976). The serotonin input to the PVN itself is very sparse (Moore et al., 1978), and lastly serotinin depletion via raphe lesions abolishes VMH hyperphagia and obesity (Coscina and Stancer, 1977). Raphe lesions also deplete PVN serotonin (Palkovits et al., 1977). In conclusion, the neurochemical identity of the afferent systems mediating food intake can not be specified.

An alternative possibility, prompted by the report of autonomic mediation of VMH hyperphagia (Powley and Opsahl, 1974), has been to look at the efferent components of this system. Hyperphagia and obesity after knife cuts beside the PVN is also abolished by vagotomy (Sawchenko, 1978). Considerable attention has therefore been given to the hypothesis that VMH hyperphagia is due to vagal disinhibition (e.g., Powley, 1977; Gold, et al., 1977), resulting in autonomic excesses, most notably hyperinsulinemia. This hypothesis receives some support from recent reports of extra-hypothalamic neurophysin pathways from the PVN to the brainstem (Swanson, 1974). Such descending pathways have also been demonstrated using horseradish peroxidase (Saper et al., 1976). A more recent report (Nilaver, et al., 1978) specifically describes an oxytocin pathway to the dorsal motor nucleus of the vagus, which could mediate food intake. Furthermore, there are reciprocal connections between the PVN and brainstem nuclei (Ricardo and Koh, 1978) which could be involved in food intake. The functional

role of these pathways has not, to date, been specifically tested. However, oxytocin and vasopressin are differentially distributed in the PVN (Defendini and Zimmerman, 1978). Oxytocin is peripherally located, in the neurons forming the "wings" of the PVN, and also more rostrally. Vasopressin is located in the medial aspects of the PVN, and more ventro-caudally. This anatomical separation tantalizingly suggests a functional difference between these cells, and their peptides, and corresponds to earlier morphological classification of the cells. Thus, the lateral(magnocellular) cells would contain oxytocin while the medial (parvocellular) cells would contain vasopressin. Based upon inspection of the series of PVN lesions and knife cuts in the present study, it appears that the medial and ventral aspects are not important for control of food intake since their destruction does not produce obesity. Conversely, destruction of the dorsolateral aspects of the nucleus produces hyperphagia and obesity. Knife cuts which spare the lateral "wings" of the nucleus do not produce the obesity either. The oxytocin cells project to the brainstem (Nilaver et al., 1978) while the vasopressin cells project to the median eminence (Vandesande et al., 1977; Zimmerman et al., 1977) and the septum (Buijs et al., 1978). Hereditary diabetes insipidus (DI), or "Brattleboro" rats have a genetic defect which obviates the production of vasopressin, hence their abnormal water regulation. However, these rats are not hyperphagic or obese (Nunez, personal communication).

A reasonable hypothesis from the above evidence is the possibility of a role for oxytocin as a transmitter which regulates food in-

take. Perhaps this could be tested by immunostaining the brains of hyperphagic, obese, selectively lateral-PVN lesioned rats, and testing whether or not the oxytocin projections to the dorsal motor nucleus of the vagus were gone. Meanwhile, existing evidence which is consistent with this hypothesis does exist.

The efferents from the PVN have been autoradiographically mapped by Conrad and Pfaff (1976). These projections run along the medial aspects of the hypothalamus and form a capsule around the VMN, thus explaining why large VMN lesions would produce hyperphagia and obesity. The map is also consistent with the knife cuts of Gold et al., (1977) and the asymmetrical (PVN x DLT) lesions of the present study. Of particular relevance, the efferents become more diffuse as they course through the tegmentum, thus accounting for the lesser magnitude of the asymmetrical lesion effect. These projections continue to descend through the tegmental reticular formation into brainstem nuclei, such as the median and dorsal raphe. The projections also continue into the pontine reticular formation and to the medial part of the nucleus of the solitary tract. These connections are also consistent with an autonomic mediation of hyperphagia and obesity.

Pfaff and Keiner (1973) in their map of estradiol-concentrating brain cells reported labeling in the PVN. The cells were located in the "wings", or magnocellular portion of the nucleus in what is now known to be the site of the oxytocin cells. In the monkey, the PVN also is labeled (Pfaff et al., 1976) and there are significant increases of the oxytocin-specific neurophysin after estradiol adminis-

tration (Robinson et al., 1976). It could be that these lateral cells are mediating the increases in circulating estrogen-stimulated neurophysin (ESN), and consequently oxytocin production. If oxytocin is a transmitter regulating food intake, then the preceding could be a mechanism whereby the well-known effects of estrogen on food intake (for review, see Wade, 1976) are mediated. This speculation brings to mind a study by Wade and Zucker (1970) in which unilateral implants of estradiol benzoate in the VMH suppressed food intake. This localization has been challenged since small VMH lesions do not abolish the anorexic effect of estrogen (e.g., Beatty et al., 1975). However, larger lesions of the VMH are reported to be effective in blocking this effect of estrogen (Nance, 1976). These larger lesions could have damaged the PVN or its efferents. Perhaps a direct test of this hypothesis would be to implant estradiol benzoate in the PVN of ovariectomized rats and observe changes in food intake. Such experiments would be useful in teasing out central effects of gonadal hormones from peripheral metabolic effects which also ultimately affect food intake and adiposity (for review see Wade and Gray, 1979). Eng et al., (1979) found that vagotomy did not abolish the weight suppressive effects of (peripheral) exogenous estrogen in ovariectomized rats. However, in order to conclusively rule out a vagal mediation of central nervous system effects of estrogen, the effect of vagotomy on the food intake suppressing effects of intradiencephalic estradiol implants would have to be tested.

An additional line of evidence supporting a role for oxytocin

brainstem pathways in food intake is the relationship between secretory cycles and activity in the PVN, and the estrous cycle (Freund-Mercier and Richard, 1977; Yukitake, 1978). Such changes are consistent with secretion of oxytocin in the afternoon of proestrus (Yukitake, 1978) which correlates well with the estrogen suppression of food intake at that time. Furthermore the oxytocin is not being released from the pituitary because neurosecretory granules in the posterior pituitary do not change over the estrous cycle (Yukitake, 1978). Thus, these secretory changes could be taking place *via* the oxytocin pathways to the brainstem. Also consistent with this model, is the report that progesterone inhibits oxytocin release (Roberts, 1971).

It is unlikely that the pituitary plays a role in PVN-lesion obesity. Although the PVN-lesioned rats grew longer (as do PVN knife-cut rats, see Gold and Kapatos, 1975), they were still obese. Furthermore, VMH lesions (Cox *et al.*, 1968) or knife cuts (Leni and Gold, 1977) still produce hyperphagia and obesity in hypophysectomized rats. Thus, a growth hormone excess is not essential for hypothalamic obesity. The posterior pituitary is also probably not involved because PVN-lesioned rats were not polydipsic. They had normal water-to-food ratios. The antidiuretic hormone (ADH) in the supraoptic nucleus is probably primarily responsible for water balance. The ADH/vasopressin in the PVN projects to the amygdala and septum, as reviewed above, and to the median eminence. The vasopressin pathways to the median eminence have been proposed as a corticotropin releasing factor, or to

participate in the release of corticotropin (ACTH) by Zimmerman et al., (1977). However, the PVN rats in the present study show none of the deficits associated with Addison's disease or adrenalectomized rats (Turner and Bagnara, 1971). PVN lesioned rats are healthy, normally active not listless, gain weight, are not polydipsic or polyuric. Furthermore, the asymmetrical (PVN x DLT) lesions which spare the PVN unilaterally, still produce hyperphagia and obesity.

Finally, the problem of interpretation of the PVN lesion's effects remains. It would be premature to interpret the present findings as revealing a new "satiety center" in the PVN. The data show only that discrete lesions of the PVN can produce hyperphagia and obesity in the absence of gonadal dysfunction, polydipsia, and hyperreactivity. The enhanced linear growth remains. It is tentatively suggested, therefore, that the PVN mediates food intake. The nature of the mechanism is probably via the autonomic nervous system. The primacy of food intake vs. metabolic changes remains an issue. Friedman and Stricker (1976) suggest that the primary effect of VMH lesions is metabolic: disruptions of nutrient processing and unavailability of fuels leading to hyperphagia. This suggestion is consistent with the primary hyperinsulinemia after knife cuts (Tannenbaum et al., 1974). VMH lesions cause a constellation of metabolic disturbances (see Bernardis 1976, for review). PVN lesions probably cause fewer disturbances, as the present data suggest. However, the question of primacy can not be answered until correlations between metabolic and food-intake changes, especially the temporal sequence of events, are

known.

CHAPTER II

CNS CONTROL OF RHYTHMS

The Hypothalamus and Behavioral Rhythms

Hypothalamic lesions, regulatory and rhythmic food intake. Lesions of the hypothalamus that disrupt the regulation of food intake also disrupt the diurnal rhythm of food intake. VMH lesions produce hyperphagia and obesity and disrupt the nocturnality of intake (Balagura and Devenport, 1970; Kakolewski et al., 1971; Becker and Kissileff, 1974; Rietveld et al., 1978). LH lesions produce aphagia and lowered body weight and increase the nocturnality of intake (Rowland, 1976). Conversely, lesions that disrupt rhythms also cause hyperphagia and obesity (Nagai et al., 1978). These results could be interpreted as evidence for a common system mediating both regulatory and rhythmic functions. Alternately, this association could be an artifact of the large hypothalamic lesions used. If the latter were the case, one would expect that PVN lesions would produce hyperphagia and obesity without abolishing rhythms.

Evidence for separate systems. In support of a separate mediation of rhythms and regulation of food intake, discrete brain damage via knife cuts beside the PVN produces hyperphagic rats without abolishing (but with some attenuation of) the nocturnal pattern of intake (Gold et al., 1975). Moreover, the magnitude of the hyperphagia seen after VMH lesions is not correlated with the degree of loss of rhyth-

micity (Rietveld et al., 1978). Also consistent with the present hypothesis is the fact that norepinephrine injected into the PVN elicits eating at night as well as eating during the day (Leibowitz, 1978b).

The disruption of nocturnality of food intake must be due to interruption of pathways from some other structure. The most likely candidate is a structure previously implicated in rhythms and a close neighbor to the PVN, the suprachiasmatic nucleus (SCN). The following section presents an overview of the rhythms literature, and then proposes a research strategy.

Adaptive significance of rhythms. Just as regulatory behaviors are important for the maintenance of internal homeostasis, rhythmic behaviors are of adaptive significance for the coupling of events in the internal environment to events in the external environment. One of the prime events in the external environment is the daily cycle of light and dark. Consequently, for most vertebrates light is a Zeitgeber (Aschoff, 1960) and the visual system plays a role in the entrainment of endogenous rhythms (for review see Moore, 1978).

It would be of adaptive value for an animal to be active and to search for food when such food is available, and when the risk of being eaten by a predator is minimal. Conversely, it would be most adaptive for the same animal to be quiescent and sleep, thus conserving energy and remaining in a safe place, when food is not available and/or predators are on the prowl. It also becomes possible for the animal, through evolution, to specialize for nocturnal activity.

In this type of situation it would also be adaptive for the animal to overeat during the active period in anticipation of the energy needs during the quiescent period. In order to accomplish these things it would be best for the animal, especially for burrowing animals, to have an endogenous "clock" so that, for example, it could anticipate day/night cyclicity, rather than be passively driven by external stimuli and continually have to check to see if night has fallen. From a physiological standpoint an internal clock could mediate the temporal sequence of metabolic and endocrine events associated with eating/satiety and activity/sleep, perhaps in an anticipatory fashion. A clock could also mediate the secretion of hormones which may have a synergistic action or whose temporal sequence of secretion may be crucial as in reproduction (Alleva et al., 1971; Brown-Grant and Raisman, 1977; Gray et al., 1978). From this evolutionary perspective it is not surprising that animals should develop endogenous rhythmic oscillators or clocks. This conclusion is supported by many observational and experimental findings(for reviews see Halberg, 1968; Pittendrigh, 1974; Rusak and Zucker, 1975) of endogenous rhythms with a circadian (approximately 24 hours, Halberg, 1960) periodicity, which persist in the absence of any external entraining cues.

Rhythms in the rodent. The laboratory rat is a nocturnal animal, doing most of its eating, drinking, and activity at night (Richter, 1965; Zucker, 1971). The endogenous nature of these rhythms is

demonstrated by their "free-funning" or continuing following blinding of the animal (Richter, 1965).

Central nervous system control of rhythms. After an extensive series of studies, Richter (1965, 1967) concluded that the clock was located in the brain since removal of all the endocrine glands did not abolish rhythms. From brain lesioning experiments, Richter further concluded that the clock was located in the hypothalamus, but he was unable to find a specific nucleus responsible for the rhythms (Richter, 1967). An earlier report by Critchlow (1963) that the SCN controlled estrous cyclicity was overlooked by researchers in biological rhythms. More recently, two laboratories have independently replicated Richter's earlier findings (Moore and Eichler, 1972; Stephan and Zucker, 1972b). These investigators used a different approach, namely the well known fact that rodent rhythms are entrained by light (Browman, 1937; Siegel, 1961; Zucker, 1971), and reasoned that a functional connection must exist between visual pathways and the clock. Surprisingly, however, interruption of the primary and accessory optic tracts did not affect the entrainment of drinking rhythms (Stephan and Zucker, 1972a). Based on these findings the existence of a direct retinohypothalamic projection was proposed. This projection was later demonstrated using amino acid autoradiography (Hendrickson et al., 1972; Moore and Lenn, 1972), and cobalt precipitation (Mason and Lincoln, 1976) to project to the SCN exclusively. The SCN is nestled on top of the optic chiasm, and the retinal input

enters from below. Thus, it is not possible to selectively cut the retino-hypothalamic tract. Instead, lesions of the SCN were performed with the expectation that entrainment would be abolished and the rhythms would freely run. The surprising finding was that the SCN lesions abolished rhythms in adrenal corticosterone (Moore and Eichler, 1972) and drinking and wheel-running activity (Stephan and Zucker, 1972b). These two experiments were the vanguard of a host of experiments implicating the SCN in the control of a great variety of rhythms: Pineal serotonin N-acetyltransferase activity (Moore and Klein, 1974), sleep (Ibuka and Kawamura, 1975; Ibuka et al., 1977; Stephan and Nunez, 1977), body temperature (Saleh et al., 1977; Stephan and Nunez, 1977), estrous cycling and sex hormones (Brown-Grant and Raisman, 1977; Raisman and Brown-Grant, 1977; Gray et al., 1978; Stetson and Watson-Whitmyre, 1976; Nunez and Stephan, 1977), drinking and activity (Nunez and Stephan, 1977; Stephan and Nunez, 1977; Rusak, 1977), and eating and drinking (Nagai et al., 1978; van den Pol and Powley, 1979).

Although the evidence strongly supports a role for the SCN in the control of rhythms, little is known concerning the neural connections mediating this control. The following section will review pertinent anatomical and experimental findings.

Anatomy and functional connectivity of the SCN. The SCN is composed of parvocellular cells (Krieg, 1932) some of which contain vasopressin and its neurophysin (Vandesande et al., 1975; Sofroniew and

Weindl, 1978), and others which contain luteinizing hormone releasing hormone (LH-RH) (Setalo et al., 1976). The role of these peptidergic cells is not known, but they may play a neuroendocrine role as suggested by the lesion studies (e.g., Brown-Grant and Raisman, 1977).

The only afferent projection to the SCN important for rhythms appears to be the retino-hypothalamic tract. The evidence for this is indirect (Stephan and Zucker, 1972a) because, for the reasons mentioned earlier, it is not possible to specifically cut the retino-hypothalamic projection. The serotonergic afferents to the SCN (Azmitia and Segal, 1978; Moore et al., 1978) are not involved in rhythms because raphe lesions did not abolish drinking or activity rhythms (Block and Zucker, 1976), plasma corticosterone rhythms (Balestrery and Moberg, 1976), or pineal serotonin N-acetyltransferase rhythms (Moore and Klein, 1974). A second visual input from the ventral nucleus of the lateral geniculate body (Swanson et al., 1974) can also be eliminated (Stephan and Zucker, 1972a) without affecting rhythms. Therefore, researchers have concentrated on the output side.

The efferents from the SCN form a dorsocaudal projection in the direction of the ventromedial and dorsomedial nuclei, and a caudal projection to the arcuate nucleus and median eminence, but the terminations of these projections are not known (Swanson and Cowan, 1975). The vasopressin cells project to the lateral septum, the medial dorsal thalmus, the lateral habenula, the posterior hypothalamus, the

interpeduncular nucleus, and the nucleus of the solitary tract (Sofroniew and Weindl, 1978). The caudal connections of the SCN appear to be important for the neuroendocrine rhythms. Retrochiasmatic knife cuts severing these fibers are equivalent to lesions of the SCN in abolishing adrenal corticosterone rhythms (Moore and Eichler, 1972), and pineal N-acetyl-transferase rhythms (Moore and Klein, 1974). Neural components mediating this latter rhythm may run in the medial forebrain bundle (Moore and Klein, 1974). Anterior hypothalamic and anterior periventricular connections (Conrad and Pfaff, 1976; Swanson, 1976) are not involved since knife cuts rostral to the chiasm had no effect on rhythms (Moore and Eichler, 1972; Moore and Klein, 1974; Nunez and Stephan, 1977).

The connections mediating drinking, eating, and activity rhythms have, however, yet to be specified. Neither retrochiasmatic, lateral, or anterior knife cuts abolish eating and drinking rhythms (Nunez and Stephan, 1977). Only a partial isolation of the SCN *via* simultaneous interruption of lateral, caudal, and dorsal efferents with a bayonet knife cut abolished these rhythms (Nunez and Stephan, 1977; Dark, in press, 1979). Nunez and Stephan (1977) proposed that the caudal efferents primarily mediate hormonal rhythms and that behavioral rhythms are mediated by separate, but undetermined, possibly diffuse, pathways. The role of the dorsal efferents *per se* has not been tested to date.

Research strategy. The role of dorsal efferents from the SCN has not

been specifically tested. Horizontal knife cuts are proposed to address this question. Also the role of lateral connections needs to be re-examined using parasagittal knife cuts that extend higher from the base of the brain than those used by Nunez and Stephan (1977), i.e., 3 mm versus their 1 mm cuts.

The issue of the specificity of the PVN lesions' effect on regulatory, but not rhythmic food intake will also be investigated in this section. PVN-lesioned rats, though hyperphagic and obese, should have nocturnal patterns of intake and activity comparable to sham-operated rats.

Methods

Subjects. The general surgical procedure was also as reported in Chapter I. Knife cuts were made using a retracting wire knife (Gold et al., 1973) cuts were made in the parasagittal and horizontal planes. The parasagittal knife cuts were placed along the lateral border of the SCN. The coordinates were: anterior 9.0 mm from the ear bar, lateral 0.5 mm, the wire was extended at 7.0 mm below the dura. The knife assembly with the wire extended was lowered to the base of the brain, raised 3.0 mm, the wire retracted, and the entire assembly removed from the brain. The length of the wire was 3.0 mm. For the horizontal knife cuts, the knife assembly was mounted on a rotator. Using the same coordinates, the wire was again extended at 7.0 mm below dura, but this time the knife was rotated 90 degrees to the right, returned to center, rotated 90 degrees to the left, and

returned to center. The wire was then retracted and the knife assembly removed from the brain.

Measures. The body weight, intake, and vaginal smear data were taken as in Chapter I. In addition, twice-daily readings were taken at the times of changeover in the light/dark cycle. Some of the rats in Chapter I were tested in Chapter II. Some of the rats were housed in Wahman activity wheels and had Richter tubes so that activity and drinking could be sampled twice daily with minimal disturbance to the rat. When these day/night samples suggested a disruption of rhythms, i.e., nocturnality was disrupted, rats were placed in an isolated, controlled environment with cycling lights, and their activity in wheels, and their eating was continuously monitored using an Esterline-Angus event recorder. The technique was that turning of the activity wheel tripped a microswitch wired to the event recorder. Similarly, eating patterns were monitored using an "eatometer" (Rowland, 1976) designed so that the rat had to deflect a metal arm, thus tripping a microswitch, in order to eat. All recording equipment was in a separate room. The resultant records would then reveal, upon visual inspection, the existence of endogenous rhythms and entrainment to the light/dark cycle, as well as eating/activity patterns. In some cases, the endogenous nature of the rhythms was further tested by placing the rats under constant dim light (approximately 2 ft-candles) and continuing to monitor eating and activity on the Esterline-Angus recorder.

Histology. Brains were processed and analyzed as in Chapter I. Representative knife cuts are shown in Figures 6 and 7.

Statistics. Where appropriate, data was statistically analyzed as in Chapter I. For the Esterline-Angus recordings, visual inspection was sufficient to determine the presence of rhythms.

Results

PVN lesions. The nocturnality of intakes was sampled for some of the lesioned rats used in Chapter I. Five rats with partial PVN lesions and 2 rats with total PVN lesions were sampled over 3 days and the results pooled for comparison with 7 sham-lesioned rats. The nocturnality of food (69.2%) and water (77.5%) in PVN lesioned rats was not significantly reduced compared to shams (70.8%, 80.4%) ($p > .10$; $t = .41, .66$; $df = 12$). These data are summarized in Figure 8. Figure 9 summarizes the activity and % of nocturnality data. Continuous activity and eating records were taken on two rats pre- and post-lesions aimed at the PVN. A representative continuous record is shown in Figure 10. Both rats had rhythms comparable to controls'. PV-1 had damage to 71% of the nucleus. PV-2 was a total PVN lesion. After the continuous recording, the rats were individually housed and their body weight gain over 11 days recorded. PV-1 gained 2.8 g/d vs. 6.5 g/d for PV-2. PV-1 was not obese, but PV-2 was (obesity indices = 315, 347.6, respectively).

Fig. 6. Representative horizontal knife cut below the PVN. The cuts are represented by solid lines extending laterally from the third ventricle.

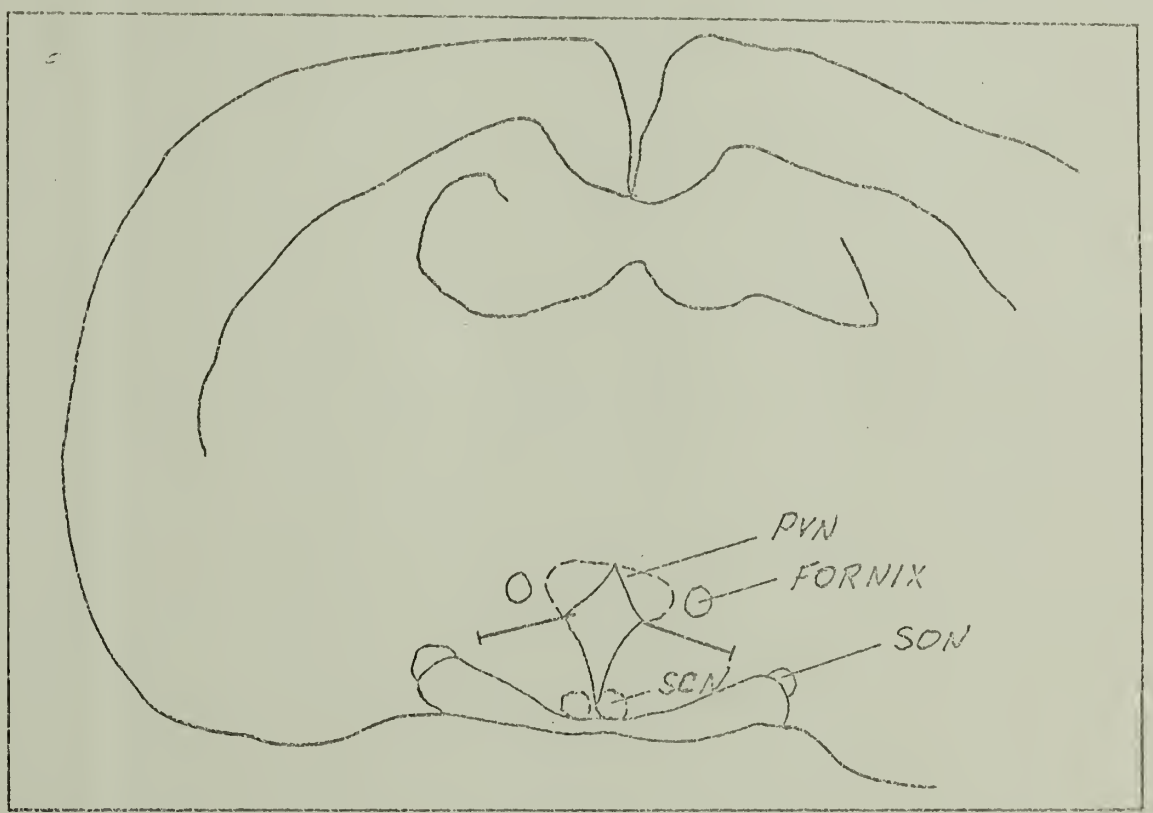

PVN
FORNIX
SON
SCN

Fig. 7. Representative SCN biparasagittal knife cuts. The cuts are represented as solid lines extending from the base of the brain.

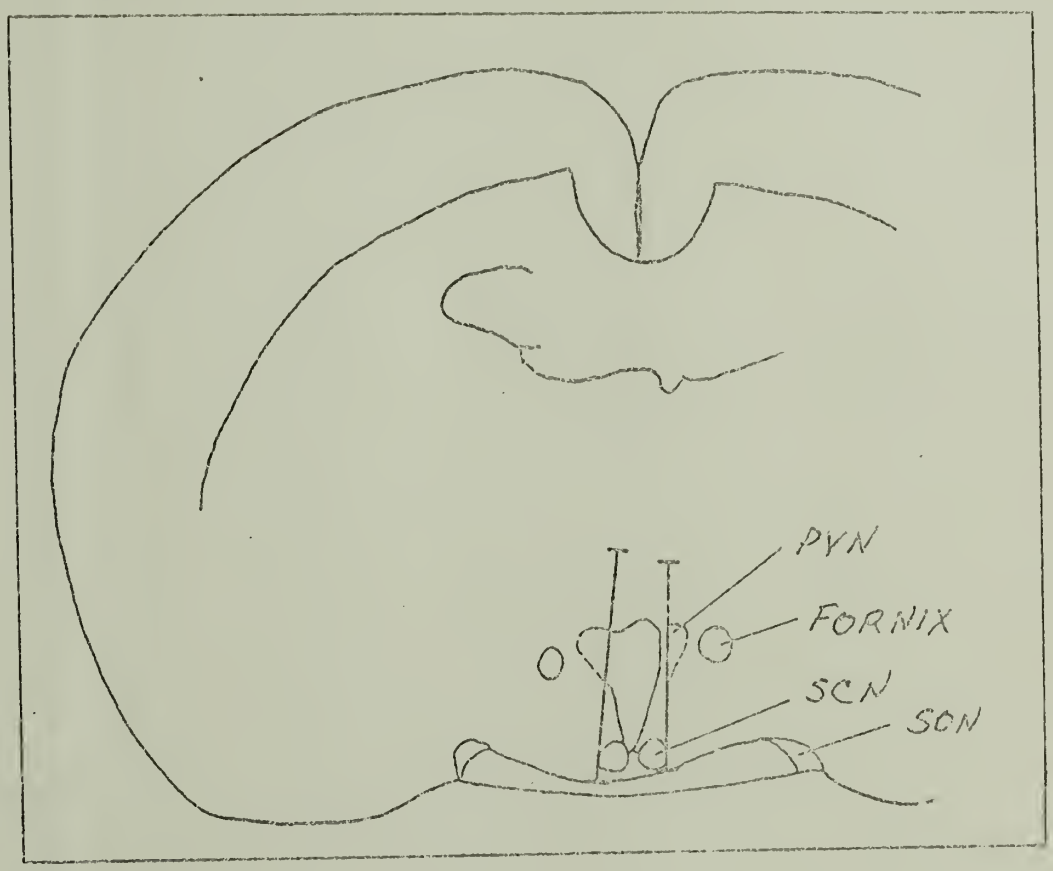
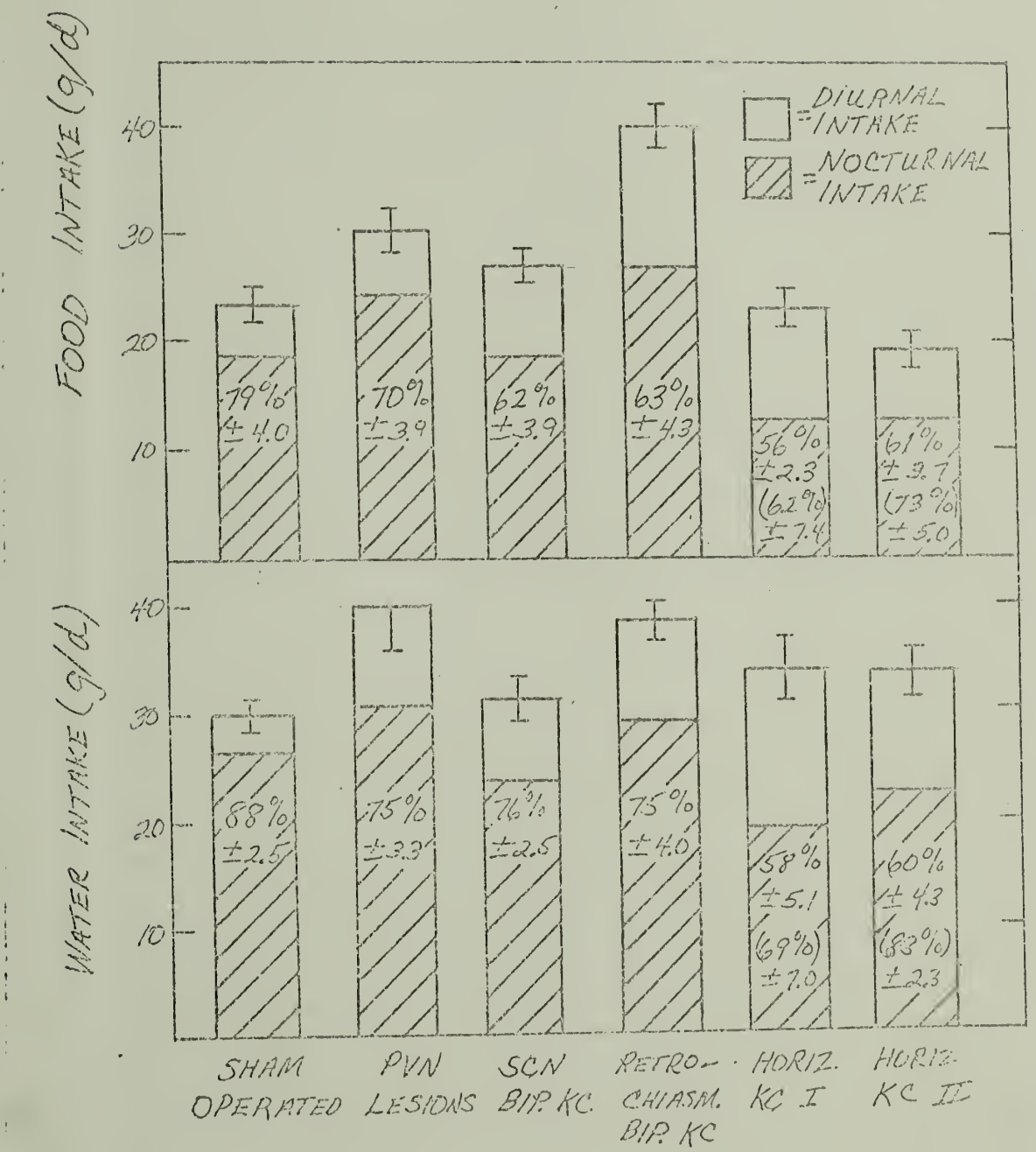

PVN
FORNIX
SCN
SON

Fig. 8. Nocturnality of food and water intake. Histograms represent the mean $\pm$ SEM intakes. Hatched area represents proportion of intake taken at night. Percentages refer to percent of intake that is nocturnal (Mean $\pm$ SEM).


FOOD INTAKE (g/d)
WATER INTAKE (g/d)
DIURNAL = INTAKE
NOCTURNAL = INTAKE
40
30
20
10
79% ±4.0
70% ±3.9
62% ±3.9
63% ±4.3
56% ±2.3
(62%) ±7.4
61% ±3.7
(73%) ±5.0
88% ±2.5
75% ±3.3
76% ±2.5
75% ±4.0
58% ±5.1
(69%) ±7.0
60% ±4.3
(83%) ±2.3
SHAM OPERATED
PVN LESIONS
SCN BIP. KC.
RETRO-CHIASM. BIP. KC
HORIZ. KC I
HORIZ. KC II

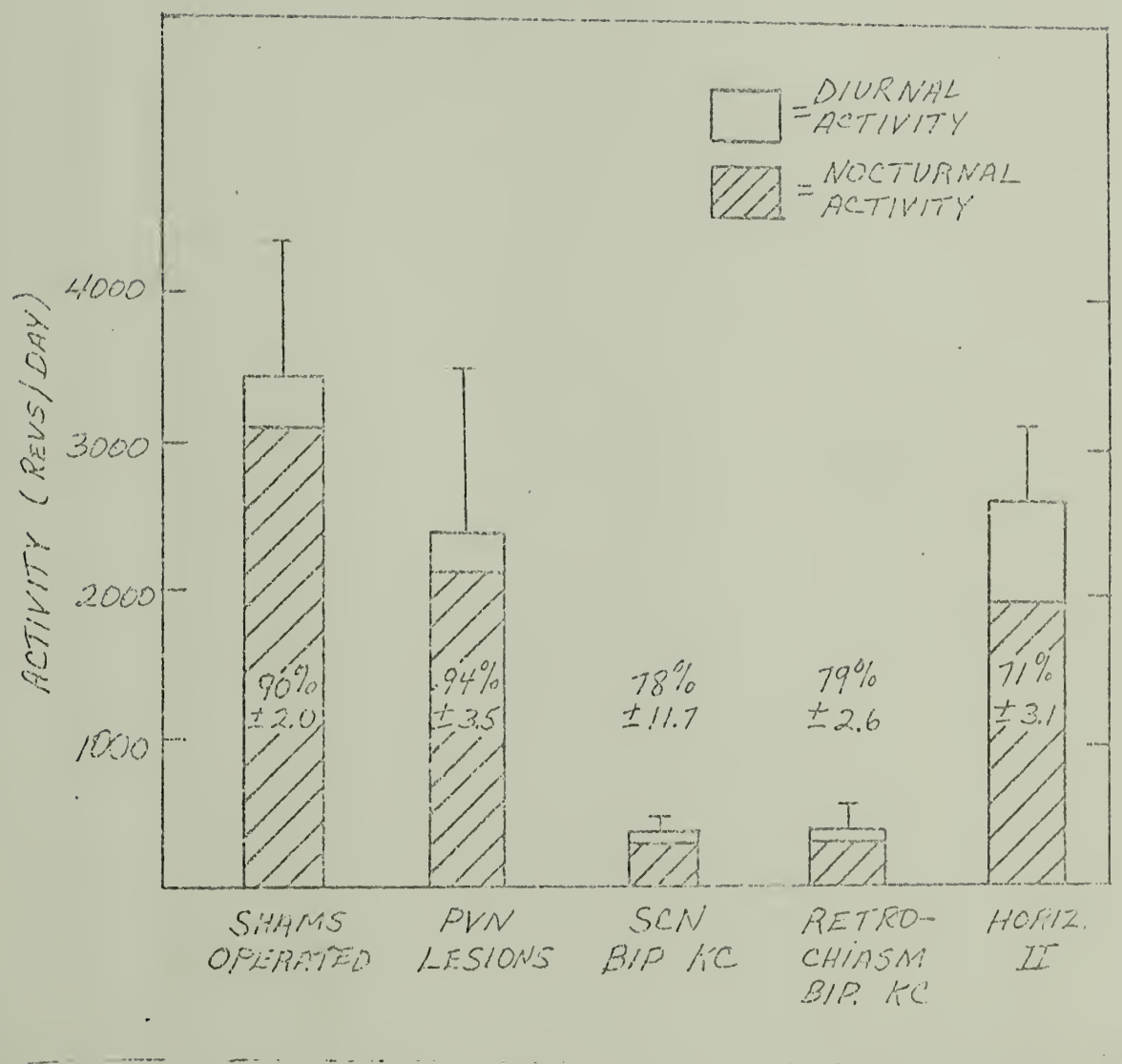


Fig. 9. Nocturnality of running wheel activity. Histograms represent daily (24 h4.) wheel revolutions. Hatched area represents nocturnal activity. Percentages refer to percent of activity that is nocturnal.

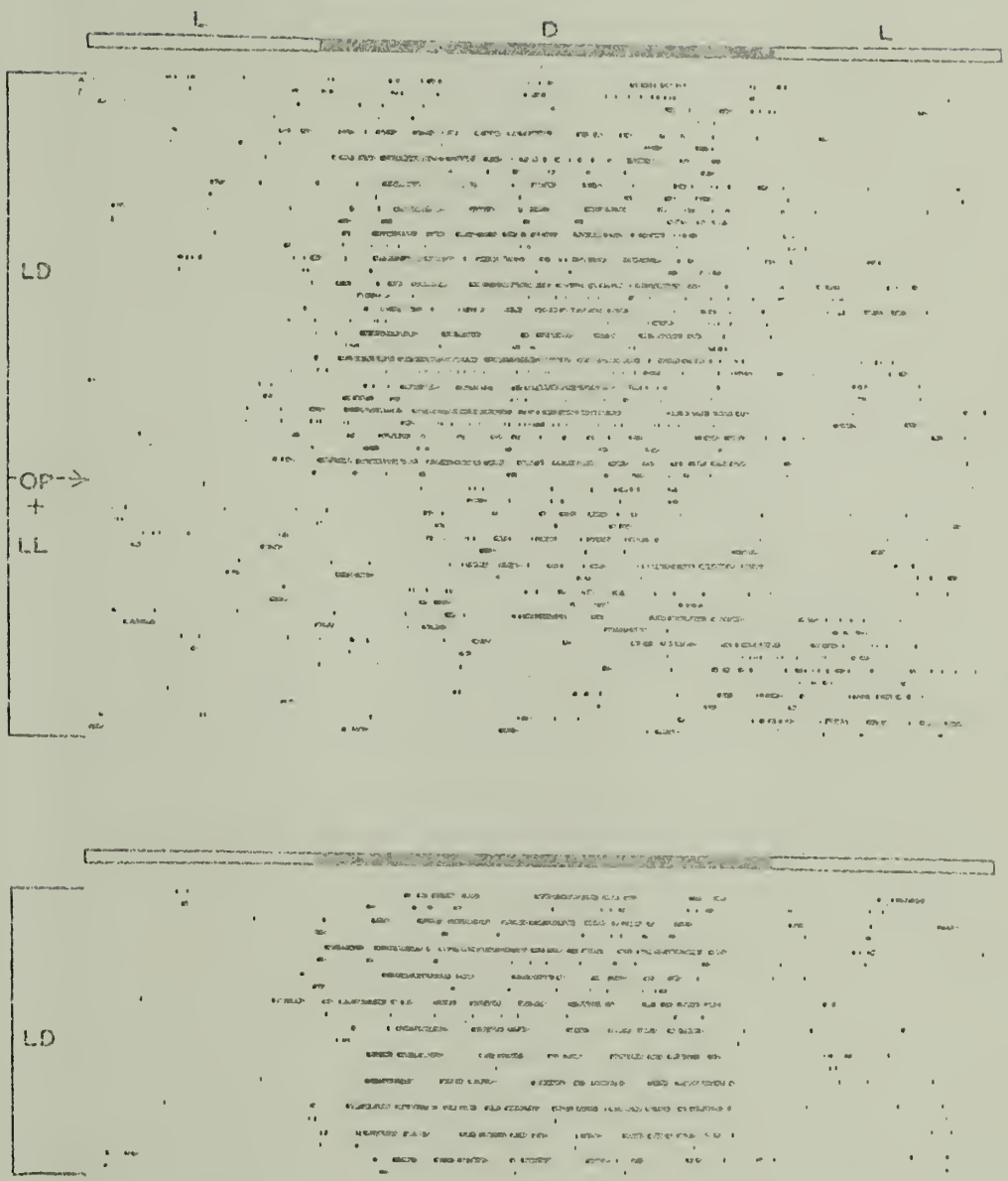


Fig. 10. Continuous record for a total PVN lesioned rat. Activity (A) and feeding (F) for each day are shown together. Break in the figure represents days of data lost due to equipment failure. L = lights on; D = lights off; LD = cycling lights; LL = constant dim light; OP = operation.

Knife cuts.

Bilateral parasagittal cuts. Based on histology, these cuts fell into two groups, with 3 rats each. One group had cuts beside the SCN. The data for this group is shown in Figure 8. No continuous records are available for this group. The other group had cuts which began in the retrochiasmatic area. As the representative record (Fig. 11) shows the rats with retrochiasmatic cuts had activity rhythms. The rats with cuts in the retrochiasmatic area, however, showed increased weight gain, obesity (obesity indices of 365-385, weight gains of 7.2-12.4) and a reduction in activity, but running rhythms were nevertheless present. The PVN did not stain in brain sections from this group. The rats activity rhythms were similar to shams when subjected to a 12 hour phase-shift of the light-dark cycle, and to constant dim light (i.e., they were free-running with a tau, or periodicity of approximately 24 hours). The eating rhythms of these rats were either abolished or severely disrupted. Inspection of the records shows a reduction of eating at light onset which was probably under exogenous control since it was not seen under constant dim light. Visual inspection also suggests the presence of ultradian components, but further data analysis would be needed.

One rat, #272 was different from the above description in terms of rhythms (Fig. 12). After 25 days on a reversed light-dark cycle, both eating and wheel running (although of low activity) were diurnal! In the constant dim light, the eating rhythm was not evident. However, when returned to cycling lights, this rat showed an entrainment

Fig. 11. Continuous post-operative record for a retrochiasmatic parasagittal knife-cut rat. Top record shows activity. Bottom record shows feeding. Note absence of a feeding rhythm despite a normal running rhythm. PS = phase shifted from a reversed light cycle. Other abbreviations as in Fig. 10.

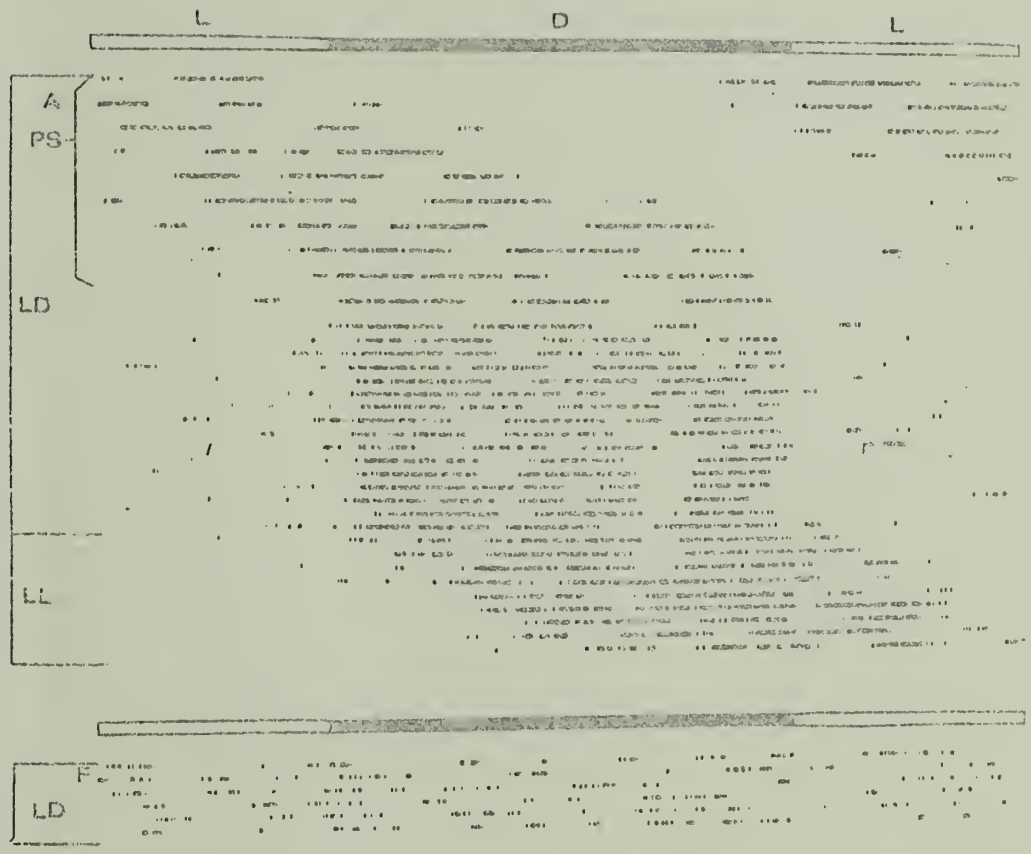
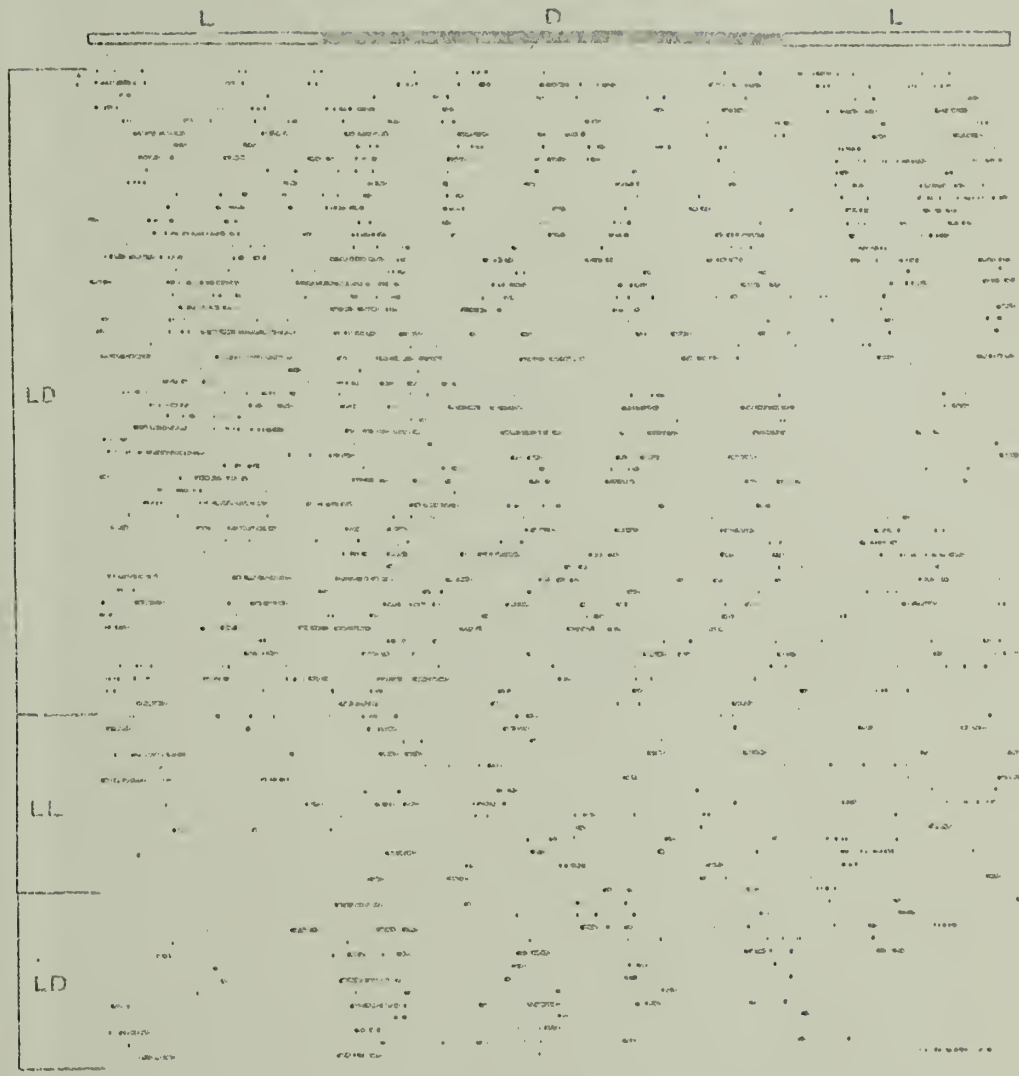

L
D
L
A
PS
LD
LL
LD

Fig. 12. Continuous post-operative activity (A) and feeding (F) records for an SCN knife-cut isolation rat. Abbreviations as in Fig. 10.


L
D
L
LD
LL
LD

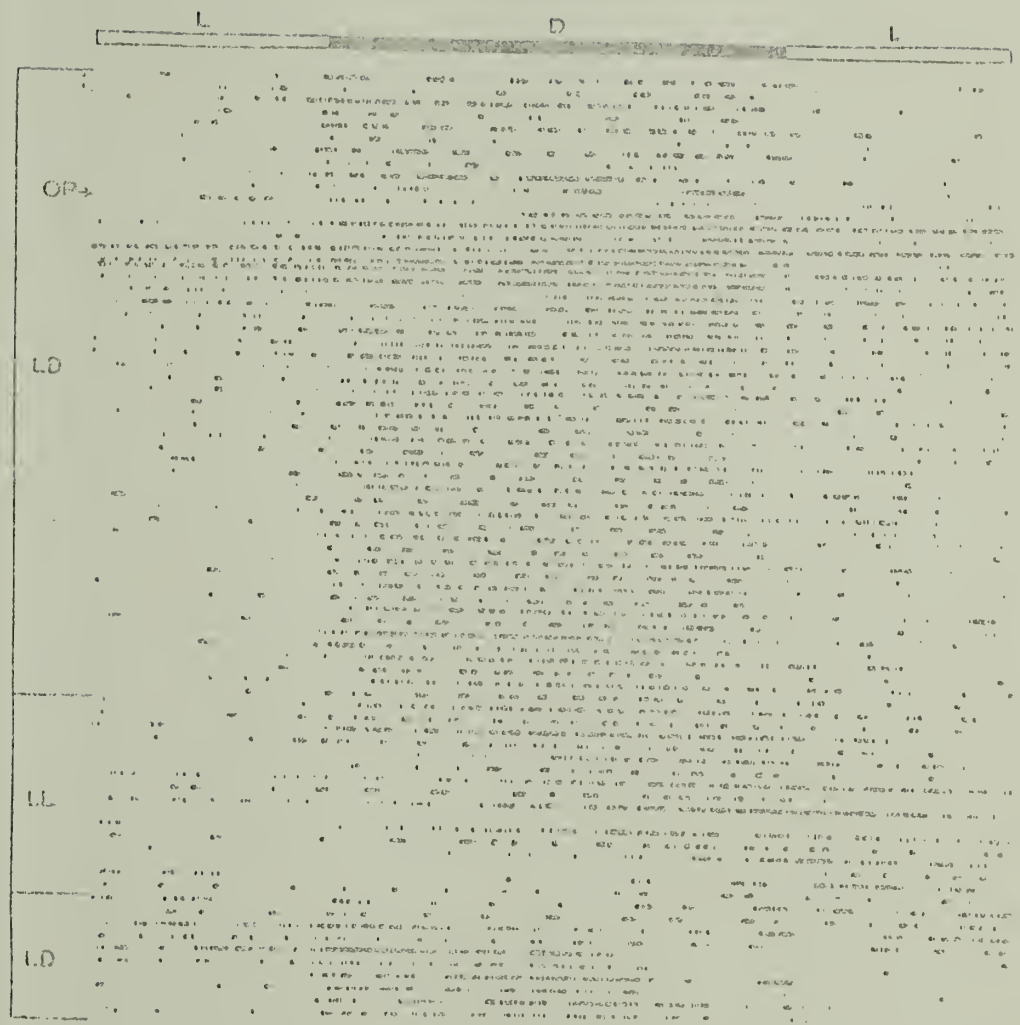
of the eating pattern, perhaps driven by the light offset. Upon histological analysis, this rat turned out to have an isolation of the SCN due to fortuitous neural damage.

Horizontal knife cuts. Based on preliminary day/night readings, horizontal knife cuts appeared to disrupt rhythms (Fig. 8). However, when these rats were tested at a later date, they had recovered their rhythms. Unfortunately, no continuous recordings were taken on those rats. Accordingly, the horizontal knife cuts were repeated. In this replication, surgery produced a transient disruption of activity and eating rhythms, which as seen in the representative record (Fig. 13) returned to normal a week post surgery. Although these rats then had rhythms entrained to the light cycle, they had more bouts of activity and eating in the light when compared to their own pre-operative records. Thus, they were less nocturnal. Under constant dim light, these rats had free-running rhythms of activity with a tau of approximately 24 hours. In 2 of the 3 rats (H-1, H-3) there was a dissociation of eating and wheel running. The eating rhythm was less well defined and showed a bimodal distribution over the 24 hour period. Upon return to cycling lights, eating and activity were again entrained to the light cycle.

Discussion

PVN lesioned rats do not differ from shams in their nocturnality of intakes. Thus, the hyperphagia after PVN lesions is primarily noc-

Fig. 13. Continuous activity (A) and feeding (F) for a horizontal knife-cut rat. Abbreviations as in Fig. 10.


L
D
L
OP→
LD
LL
LD

turnal, suggesting no disruption of rhythms. This suggestion is supported by the continuous records of rats PV-1 and PV-2. Therefore, the hypothesis of separate systems mediating diurnal rhythms *vs*. regulatory intakes is demonstrated.

The horizontal knife cuts severing dorsal efferents from the SCN temporarily disrupted rhythms, but the rats soon recover. Therefore the dorsal efferents from the SCN are not solely responsible for the mediation of rhythms. These connections play some role, however, because the observed disruption of rhythms is specific to horizontal cuts and not seen after sham or control knife cuts.

The SCN parasagittal knife cuts severing the lateral efferents from the nucleus did not disrupt rhythms. Taken together with the horizontal cuts, these results are congruent with those of Nunez and Stephan (1977) and Dark (in press, 1979). These investigators reported that only simultaneous interruption of all dorsal, caudal, and lateral efferents abolished rhythms permanently. Thus the pathways mediating rhythms are probably very diffuse. This conclusion is consistent with the known diffuse projections of SCN efferents (Swanson and Cowan, 1975; Sofroniew and Weindl, 1978).

PVN lesions apparently affect regulatory but not circadian constraints on eating and activity. Such a conclusion is supported by the report that norepinephrine injected into the PVN elicits eating during the day or night (Leibowitz, 1978b). The rats with parasagittal cuts starting at retrochiasmatic levels, were hyperphagic and obese with no eating rhythms. However, although hypoactive, they did

most of their running at night, and were also nocturnal drinkers. Since the PVN did not stain in the histology of these rats, damage to efferents followed by degeneration in PVN can be inferred. This can be contrasted with the SCN parasagittal cuts which spared the lateral "wings" of the PVN. These cuts produced neither hyperphagia nor disruption of feeding or running rhythms. The damage caused by the large retrochiasmatic parasagittal cuts is not necessarily equivalent to that of discrete PVN lesions. Because the cuts are so long (3 mm) they cut a large number of fibers in the basal hypothalamus. This could cause complex neuroendocrine and metabolic disturbances which could contribute to the observed hyperphagia and obesity. Subtle differences in the anter-posterior extent of the cuts are probably of relevance. The cuts that produced hyperphagia and obesity were more caudal. The cuts also produced hypoactivity which no doubt contributed to the obesity. This multiple factor explanation for the obesity receives support from Hennessy and Grossman's (1967) report that hyperphagia and obesity after coronal knife cuts in the posterior hypothalamus were attenuated by daily estradiol injections. PVN lesioned rats have normal estrous cycling, thus the lesser magnitude of the hyperphagia and obesity is consistent with normal ovarian function. Hormonal disruptions, particularly hyperinsulinemia could account for the dissociation of eating from other rhythms in the rats of the present experiment which became hyperphagic and obese. In support of such a notion, insulin injections can mimic the disruption of eating rhythms seen in VMH rats (Larue-Achagiotis and

Le Magnen, 1979).

The rat with the fortuitous isolation of the SCN (#272), became diurnal. This result, though surprising, is not unique. Similar inverted (light-active) records have been obtained after surgical isolation of the SCN (Richter, 1978) and electrolytic SCN lesions (Richter, 1978; Nunez, personal communication). Richter (1978) attributed the diurnal activity to damage extending beyond the SCN itself, as he only obtained the inversion by large isolations or lesions of the SCN. Richter concluded that the inverted patterns were due to elimination of the clock in the SCN. However, the light-active pattern was not eliminated by blinding the rats. Thus after the destruction of the clock in the SCN, some other bodily function is mediating the rhythms observed by Richter. Despite their loss of circadian rhythms, rats with SCN lesions exhibit anticipatory wheel running in response to a restricted (access to food at 24-hour intervals) feeding schedule (Stephan et al., 1978). This finding suggests that either circadian oscillators outside the SCN can be entrained by restricted feeding schedules, or anticipatory running is due to an "hourglass" type of clock which must be reset daily.

Consistent with the present hypothesis of separate systems, Richter (1978) reported that the rats with "the shift to light activity had no detectable ... (disruption) of any of the metabolic functions (total daily running, daily food and water intakes, body weight)." Thus the clock function is independent of any regulatory functions of the hypothalamus. After destruction of the clock, some

metabolic functions may take over a synchronizing role. Possible candidates are the blood glucose and insulin levels which are reported to show a circadian fluctuation (Gagliardino and Hernandez, 1971).

GENERAL DISCUSSION

The present results support the hypothesis of separate neural systems mediating regulatory and rhythmic aspects of behavior. This conclusion is based on the findings that PVN lesions produce hyperphagia and obesity without disrupting rhythms, estrous cycling, or reactivity.

The relationship between regulatory and circadian constraints on eating remains open to speculation. The normal rat is hyperphagic during the night and hypophagic during the day. These behavioral changes are reflected in metabolic fluctuations also (Le Magnen et al., 1973). However, the normal rat would not show a circadian fluctuation if it were only driven by metabolic events. If such were the case, one would expect that the rat would eat periodically during the day and night in response to energy needs. Clearly this is not the case. It is likely that some clock drives these metabolic functions. The SCN is the most likely candidate because after SCN damage circadian eating rhythms disappear (van den Pol and Powley, 1979). However, there is no regulatory disruption because SCN-lesioned rats do not become hyperphagic, hypoactive, or obese. Conversely, specific damage to the PVN in the present study, ventral noradrenergic bundle lesions (Ahlskog and Hoebel, 1973), knife cuts along the PVN (Gold et al., 1975), intrahypothalamic gold thioglucose (GTG) (Reitveld et al., 1979), bilateral DLT lesions (Peters, et al., 1979, submitted) all produce hyperphagia which is still

clearly nocturnal, even though this nocturnality is often dampened. This suggests a separate, food intake regulating system. VMH lesions (e.g., Kakolewski et al., 1971) probably damage components of both regulatory and rhythms systems.

One would expect that regulatory and rhythmic food intake systems must somewhere interact, sharing a common neural output and at least a final common motor system, as the regulated behavior is common to the two. It is not yet known, however, where such interaction could take place, and hence an additional food intake regulatory brain site is to be discovered.

Hepatic vagotomy, although altering the normal daily rhythm of food intake, does not affect the entrainment of eating to the light/dark cycle (Sawchenko et al., 1979, submitted). Liver signals are probably also important for food intake (Sawchenko and Friedman, 1979). The clock in the SCN could modulate the relative importance of liver signals. For example, the clock could suppress liver signals of nutrient excess, thus mediating nocturnal hyperphagia. Removal of the clock via a lesion would allow liver signals to take precedence, thus the rat would eat throughout the day in response to hourly energy fluctuations. Daytime hypophagia could be similarly interpreted, with the clock suppressing nutrient depletion signals. However, liver signals can not be the only mechanism.

The central neurocircuitry of the PVN and the SCN and how they respectively affect regulatory and diurnal eating remains unresolved. The SCN can not act directly on the PVN because PVN lesions

or horizontal cuts do not disrupt eating rhythms and vice versa. The SCN could be acting on VMN cells which are separate from gluco-receptor cells (Rietveld et al., 1979). These VMN cells could then mediate some of the circadian metabolic fluctuations. It is also possible that PVN cells could send collaterals to the VMN, because PVN efferents to the brainstem pass in close proximity to the VMN (Conrad and Pfaff, 1976). These conjectures remain the subject of future investigations.

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