Pharmacology Biochemistry & Behavior, Vol. 8, pp. 163–175. Printed in the U.S.A.

Paraventricular Nucleus: A Primary Site Mediating Adrenergic Stimulation of Feeding and Drinking[1]

SARAH FRYER LEIBOWITZ

The Rockefeller University, New York, NY 10021

(Received 5 July 1977)

LEIBOWITZ, S. F. *Paraventricular nucleus: a primary site mediating adrenergic stimulation of feeding and drinking.* PHARMAC. BIOCHEM. BEHAV. 8(2) 163–175, 1978. – Central injection of norepinephrine (NE) has been found to elicit preprandial drinking and feeding responses in the satiated rat. In the present study, 35 different brain areas, in over 500 rats, were examined to localize the precise region of NE sensitivity. Essentially all sites outside the hypothalamus, as well as in the lateral portion of the hypothalamus, were relatively or totally unresponsive to NE. In the medial hypothalamic area, the paraventricular nucleus (PVN) was clearly distinguished as the most effective site for initiating both feeding and drinking with noradrenergic activation in the satiated animal. Sites greater than 0.5 mm rostral, caudal, dorsal, ventral or lateral to this nucleus yielded significantly smaller effects. In mildly hungry rats, NE was found to potentiate the ongoing feeding response, and anatomical analyses of this phenomenon showed the PVN to be most responsive, with a smaller but reliable potentiation occurring along the periventricular hypothalamus adjacent to the third ventricle. Norepinephrine injected into the lateral perifornical hypothalamic area actually produced a suppression of feeding in these hungry animals. These findings, together with results from other studies, converge on the medial PVN region as being a key link in the process of increased food and water consumption associated with increased noradrenergic activity.

Drinking behavior Feeding behavior Hypothalamus Norepinephrine Autoradiography Paraventricular nucleus Adrenergic receptors

ADRENERGIC agonists, when administered directly into the hypothalamus of rats, have been shown to have profound effects on ingestive behavior (for review, see [30, 41, 47]). In satiated animals, these agonists have been found to produce a vigorous feeding response, apparently mediated by alpha receptors [6, 22, 23, 43, 44, 74], and a preprandial drinking response which involves alpha and beta receptors [43,44]. In hungry animals, both a potentiation and a suppression of feeding effect have been described, depending on site of injection (see DISCUSSION). As in satiated rats, the potentiation effect in hungry rats is mediated by alpha receptors [47,68]; the suppressive effect, in contrast, is mediated by beta receptors [21, 37, 47, 48, 56, 57].

These observations on the effects of central adrenergic stimulation on food and water intake have inspired researchers to examine the question of whether adrenergic receptor mechanisms in the brain have a physiological function in the regulation of natural ingestive behavior. In support of this suggestion, recent studies have been able to demonstrate reliable changes in feeding (either a potentiation or a suppression) with quite low doses of the adrenergic agonists, in the low ng range [40, 44, 49, 68]. Moreover, there is additional evidence that a rat's normal feeding behavior can be predictably altered by drugs that act indirectly through endogenous adrenergic mechanisms, either by releasing the adrenergic neurotransmitter from central nerve endings or by blocking adrenergic receptor sites (for review, see [47]). In addition, close examination of adrenergically induced ingestive behaviors in satiated rats has revealed remarkable similarities between these elicited behaviors and a rat's normal ingestive behaviors [43]. The eating response triggered by hypothalamic adrenergic stimulation is similar in magnitude and duration to a rat's normal meal. Furthermore, adrenergic stimulation produces a drinking response which, as under normal conditions, immediately precedes the meal and is positively correlated in magnitude with the size of the subsequent meal.

In light of this evidence for the existence of central adrenergic mechanisms controlling ingestive behavior, the present study was focused on the problem of determining where in the brain these adrenergic mechanisms might exist. With regard to the feeding response elicited by norepinephrine in satiated rats, Coury [11] obtained evidence suggesting a possible link between this phenomenon and the Papez circuit. Booth [5], however, pointed out a specific site in the brain that he found to be particularly responsive to norepinephrine. This site, which is located just next to the fornix (approximately 1 mm lateral to midline) at the anterior hypothalamic level, has been confirmed by other

[1] This research was supported by NIH research grant MH 22879 and by funds from the Whitehall Foundation. The author gratefully acknowledges the excellent technical assistance of Mrs. Ruth Hechinger and Messrs. Kevin Chang and Norman Hammer. The author wishes to thank Dr. Anthony Sclafani for reading the manuscript and providing helpful comments.

0091-3057/78/0802-0163$01.35

investigators [14, 43, 74] to be a particularly sensitive area, in contrast to more lateral and posterior sites in the lateral hypothalamus, where catecholamines and amphetamine have actually been found to cause a suppression of feeding [37, 38, 45, 48, 56, 57]. In general, the evidence appears to favor the view that the medial hypothalamus is considerably more sensitive than the lateral hypothalamus with respect to noradrenergic stimulation of feeding [14, 22, 38].

This evidence has laid the foundation for the present series of experiments, in which the effects on ingestive behavior of centrally administered norepinephrine were examined as a function of injection site in over 500 rats. Thirty-five different areas of the brain, throughout the mesencephalon, diencephalon, and telencephalon were investigated. The results of these tests have clearly distinguished the hypothalamic paraventricular nucleus as the most effective site for potentiating feeding and preprandial drinking with central adrenergic stimulation.

Some of the results described in this article were presented in preliminary form at the Third International Congress on Catecholamines in 1973 [40] and the 45th Annual Meeting of the Eastern Psychological Association in 1974 [42].

METHOD

Animals

The animals were 566 male albino Sprague-Dawley rats weighing 350–400 g at the start of the experiment. They were housed individually and maintained and tested, in their home cages, on Purina lab chow pellets and tap water. Most rats were tested while food- and water-satiated, although in one experiment the animals were tested after mild overnight deprivation during which 80% of their normal food intake was made available. The animals were kept on a constant light-dark cycle, with the 12-hr light phase beginning at 6:00 a.m. followed by a 12-hr dark phase.

Surgery

Each rat was stereotaxically implanted under Nembutal anesthesia with a chronic unilateral cannula. These cannulas (made from 23- or 26-gauge hypodermic needles), which had a screw-on protective cap and an inner stylette as described elsewhere [43], were aimed at one of 35 different regions in the brain and were fixed in place on the top of the skull with acrylic cement and stainless steel hooks penetrating the bone. All drugs were injected directly into the brain via these implanted cannulas.

General Test Procedure

The rats were given 2 weeks of postoperative recovery before testing was started. During this period, they were frequently handled and mock-injected in order to adapt them to the test procedure. The drug tests were conducted in the morning (approximately 10:00 a.m.) every 2 or 3 days. During a 1-hr pretest period, the rats (with the exception of those tested after mild food deprivation) were given fresh food and water, to insure maximal satiation, and were handled and mock-injected at least once during this period. At the end of this pretest hour, the rats were injected with the drug vehicle (0.5 μl of sterile physiological saline) and then immediately given measured pellets and/or water. The water was made available through calibrated tubes with drinking nozzles, and measurements of water consumption were taken 10 and 30 min after injection. The measured food was placed in a corner of the cage with measurements being recorded 30 min after injection. The food lost through spillage was added to the unconsumed total. There was no apparent spillage of water. At the end of this 30-min saline control test, the rats received injections (0.5 μl) of l-norepinephrine-d-bitartrate (NE) dissolved in physiological sterile saline (0.9%). A test identical to that just described for saline alone was then carried out for the adrenergic agonist.

In one experiment, the rats were tested after mild food deprivation; that is, the test began after a 24-hr period during which only 80% of the rats' normal food intake was made available. The purpose of these tests was to examine the effect of NE in slightly hungry animals which had just initiated a meal. For this experiment, NE or saline was injected in a counterbalanced sequence (ABBA). Food and water intake were measured 30 min after injection.

Except where indicated, statistical evaluations of the results were based on a two-tailed *t*-test for dependent means. The saline baseline food intake scores for the satiated rats were generally near zero (between 0.0 and 0.4 g). The water intake scores during the first 10 min were almost always zero. For the mildly deprived rats, the meal size exhibited during the saline control test varied between 1.6 and 2.1 g.

Histology

To determine the precise placement of the brain cannulas, the rats at the termination of each experiment were sacrificed under Nembutal anesthesia and perfused with saline and a 10% buffered Formalin solution. Their brains were removed from the skull, and frozen sections of 50 μ were cut and then stained with cresyl violet. The location of the cannula tips was determined according to the atlas of König and Klippel [35].

EXPERIMENT 1: MAPPING OF NE-ELICITED FEEDING

This experiment examined the NE-elicited eating response in satiated rats as a function of the injection site in the brain. A total of 357 rats were used. Each animal was implanted with a unilateral chronic cannula aimed at a single site within the mesencephalon, diencephalon, or telencephalon. The rats were then subjected to approximately 6 tests (one every 2 or 3 days), in which saline and then NE (at a moderately high of 40 nmoles) were injected as described in the METHOD section. Only food was available during these tests, and the rat's latency to eat after injection, as well as their total food consumption after 30 min, was recorded.

Results and Discussion

A total of 35 brain areas were tested in this experiment. The sensitivity of these areas to exogenous NE, with respect to its elicitation of feeding effect in satiated animals, is diagrammatically illustrated in Fig. 1. Each area is represented by a circle with a number enclosed. Between 6 and 20 rats had cannulas aimed at a particular area, and the number within each circle indicates the magnitude of the average feeding response (in grams) produced in these rats by NE injection at that site. (Saline baseline score was

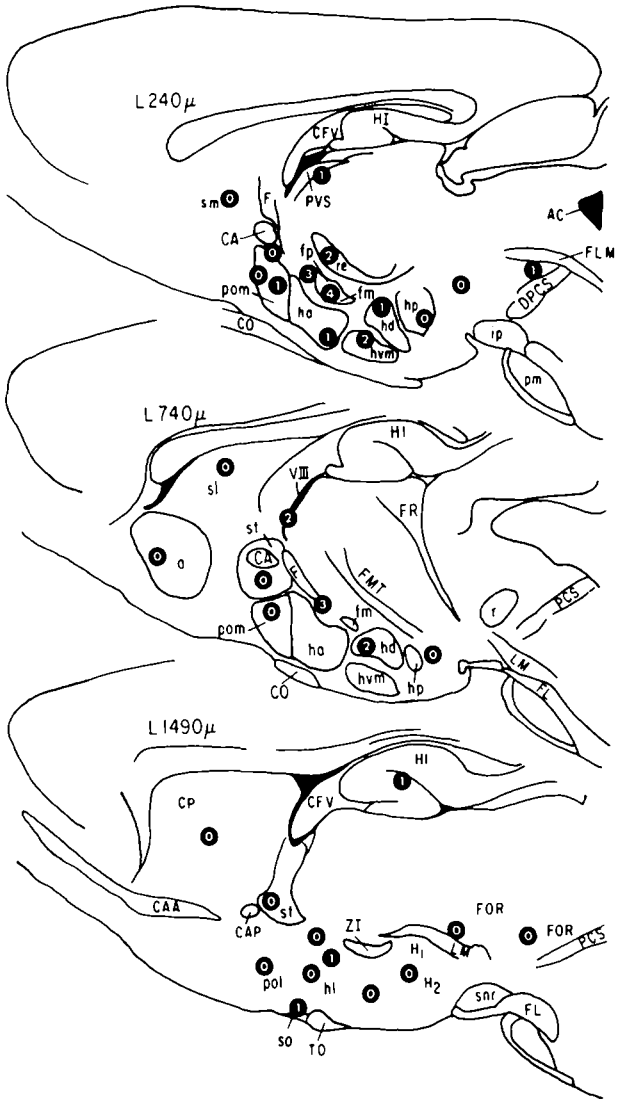

FIG. 1. Schematic representation of central injection sites for norepinephrine and its stimulatory effect on feeding behavior in satiated rats (N = 357). Each site is represented by a circle with a number enclosed. Between 6 and 20 rats had cannulas aimed at a particular area, and the number within each circle indicates the magnitude of the average feeding response (in grams) produced in these rats by norepinephrine (40 nmoles) injected at that site. (0 = 0.0 to 0.5; 1 = 0.6–1.5; 2 = 1.6 to 2.5; 3 = 2.6–3.5; 4 = 3.6 to 4.5.) Sagittal sections and abbreviations are derived from König and Klippel's atlas of the rat brain [35]. Abbreviations within hypothalamus: fm, nucleus paraventricularis pars magnocellularis; fp, nucleus paraventricularis pars parvocellularis; ha, nucleus anterior; hd, nucleus dorsomedialis; hvm, nucleus ventromedialis; hp, nucleus posterior; hl, nucleus lateralis; so, nucleus supraopticus; ZI, zona incerta; F, fornix; H_1 and H_2, Forel's field.

generally less than 0.4 g). In general, it appears from these results that the feeding response induced by centrally injected NE in satiated rats is a site-specific phenomenon, with the hypothalamic paraventricular nucleus (PVN) and its vicinity being strongly distinguished as the site of greatest sensitivity.

Essentially all sites outside the hypothalamus (tested in 183 rats) were found to be relatively or totally unresponsive to NE. The totally insensitive structures included (from anterior to posterior) the nucleus accumbens septi, caudate-putamen, medial and lateral septum, interstitial nucleus of the stria terminalis, lateral preoptic area, and caudal regions at the midbrain-hypothalamic juncture and within the medial and lateral midbrain tegmentum. (Not shown in Fig. 1 are two placements aimed at the anterior and posterior portion of the central amygdaloid nucleus. In confirmation of Grossman's earlier work [24], NE at these sites was also ineffective in initiating feeding.) A small to moderate response (reliable at least at $p<0.05$) could be observed with NE injection into the hippocampus, periventricular nucleus of the thalamus, and the third ventricle (at the level of the preoptic area). The effectiveness of these ventricular or periventricular sites is most probably due to the spread of NE to the sensitive area (see below and GENERAL DISCUSSION).

Within the hypothalamus itself (N = 174), all but two lateral sites (that is, sites lying 1.3 mm or more lateral to the midline) were found to be unresponsive to NE. The two exceptions, the supraoptic nucleus and the dorsolateral hypothalamus at the level of the PVN, yielded a small but reliable enhancement of 1 g. This pattern of lateral hypothalamic insensitivity contrasts dramatically with the effectiveness of medial hypothalamic injection of NE. While the posterior hypothalamus appeared unresponsive, a small to moderate increase of food consumption was observed after NE administration into the anterior, ventromedial, and dorsomedial regions, as well as the medial preoptic area. By far the largest and most consistent response, however, was observed after NE injection directly into the PVN. An increase of 4 g ($p<0.001$), consumed in approximately 15 min, was produced by NE in rats with cannulas aimed at this structure. This contrasts with the somewhat weaker response (1–3 g increase) produced in rats with cannulas just rostral, caudal, dorsal, ventral, or lateral to this nucleus. The injection site just lateral to the PVN, which yielded a 3 g response, represents the perifornical site (perhaps slightly caudal) originally described by Booth [5] and subsequently tested by several other investigators [14, 43, 74].

From these results, it appears that the PVN or its immediately surrounding tissue might be NE's primary site of action for eliciting feeding in satiated rats. While somewhat smaller eating responses can be observed with injection into structures outside the PVN, the gradual decrease in the magnitude of this response with increase in distance from the PVN suggests that the apparent sensitivity of other structures might be attributed to the diffiusion of NE to the PVN (see GENERAL DISCUSSION). Evidence to support this suggestion is provided by additional results showing the rats' latency to respond to NE. The longest latency (6.5 min, ranging from 3 to 10 min) characterized the eating response elicited by NE injection into structures further than 1 mm from the PVN. A shorter latency of 4.5 min (ranging from 2.5 to 8.0 min) was obtained from structures within a 1-mm radius of the PVN (see [43]), while an even shorter latency 2.5 min, ranging from 1.5 to 4.5 min) was obtained from the PVN itself. In these PVN animals, behavioral signs indicative of the onset of a meal (such as searching for or handling the food) were observed prior to this time, approximtely 1.2 min after injection. We have observed even shorter latencies (30 sec or less) in animals that were undisturbed during the NE injection procedure (that is, injected

remotely) rather than being handled during this process as is usually done when large numbers of animals are being tested. It should perhaps be noted that no water was available during these tests on food intake. The presence of water will frequently delay the feeding response, by allowing initial drinking to occur [43].

EXPERIMENT 2: FURTHER ANALYSIS OF PVN SENSITIVITY TO NE

The NE mapping results obtained in Experiment 1 clearly demonstrate the largest NE-elicited feeding response and shortest latency to respond after injection into the dorsomedial portion of the anterior hypothalamic region. While the evidence is indicative of PVN involvement in this response, the limitations of the cannula-injection technique, with regard to the extent of drug spread from the site of injection (see GENERAL DISCUSSION), preclude any firm conclusions on whether the tissue sensitive to NE lies within the boundaries of the nucleus or somewhere in its vicinity.

To provide insight into this question, additional animals were tested (N = 60; between 11 and 15 for each injection site) in which small adjustments in the cannula placements were made along the ventral and lateral border of the PVN as well as within the rostral and caudal portions of this nucleus. For these tests (identical to those of Experiment 1), a smaller, 26-gauge, cannula was used to minimize damage and drug spread at the injection site. Furthermore, only 4 tests with NE (40 nmoles) were conducted to further reduce the trauma to the brain tissue. Although these precautions would not be expected to eliminate the problems associated with the cannula-injection technique, they might facilitate the process of detecting small differences in the sensitivity to NE of various parts of the PVN and its immediate vicinity. This experiment was used further to identify the precise stereotaxic coordinates for implanting a cannula aimed at the most responsive site.

Results and Discussion

In Fig. 2, the behavioral findings of this experiment are illustrated on a frontal section of the rat brain (A5340 μ according to König and Klippel [35]) at the level of the PVN. In Fig. 3, brain photomicrographs (frontal sections) are presented showing the injection sites for 6 of the rats used in this study. The results obtained from this analysis confirm the responsiveness of the anteromedial hypothalamus to NE injection. Furthermore, they indicate that the most sensitive tissue may lie within the borders of the PVN itself, as opposed to along its lateral, ventral, or caudal edges.

As demonstrated in Figs. 2 and 3, injection sites aimed at the dorsomedial portion of the PVN (see Fig. 3a and b) yielded the largest eating response of approximately 4.2 g after NE administration. A small movement of the cannula (0.5 mm) to the dorsolateral edge of the PVN, just medial to the fornix (Fig. 3c), was apparently sufficient to reliably reduce (at $p<0.05$) the average meal size to 3.3 g. A somewhat smaller meal, 2.9 g, was generally observed in a slightly more lateral placement just on top of the fornix (Fig. 3d). (This perifornical placement is the same as that examined in our previous studies on NE-elicited ingestive behavior [43,44].) Further movement of the cannula

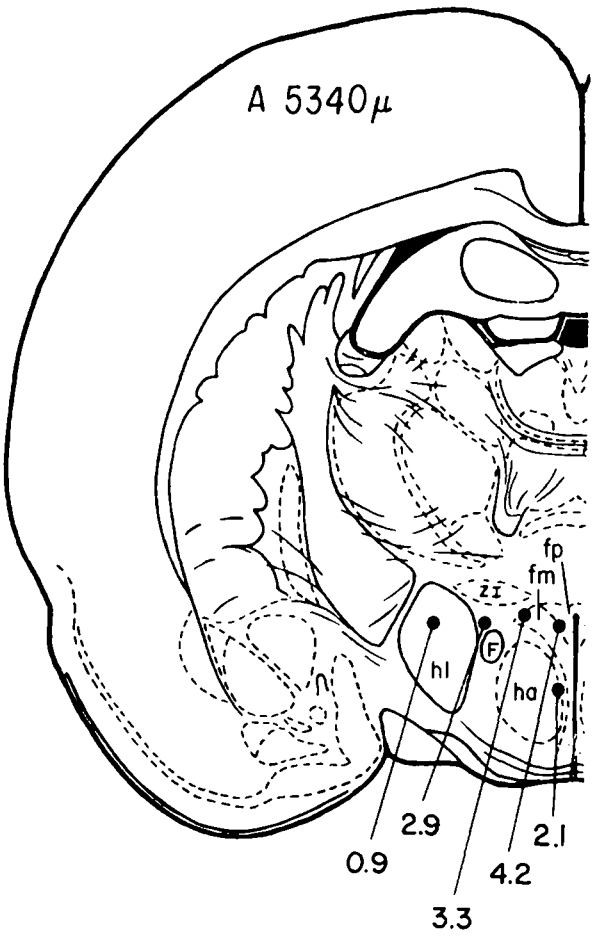

FIG. 2. Frontal diagram of the rat brain based on König and Klippel's atlas of the rat brain [35] illustrating food intake scores (grams averaged over 4 tests) obtained after norepinephrine injection into 5 sites at the level of the paraventricular nucleus (A 5340 μ). Between 11 and 15 rats had cannulas aimed at each site indicated by a filled circle (•). All feeding scores reached statistical significance at least at $p<0.01$, with the exception of 0.9 g in the lateral hypothalamus, which was statistically unreliable. The food intake score for the dorsomedial portion of the paraventricular nucleus (4.2 g) was reliably greater (at least at $p<0.05$, as determined via independent t-test comparisons) than all other scores presented in this figure. For abbreviations, see legend to Fig. 1.

lateral to this site (Fig. 3e) or ventral to the PVN (Fig. 3f) revealed a dramatic decrease in responsiveness, with meal size averaging 0.9 and 2.1 g, respectively. These results clearly distinguish the PVN, apparently its dorsomedial portion, as being the most sensitive area for stimulation of feeding with noradrenergic activation. Further dissection of the results along the anterior-posterior plane indicates that injection sites within the rostral or middle portion of the PVN are slightly more responsive than those lying along its caudal edge. Animals with cannulas terminating at levels A5660 μ and A5340 μ [35], within or just lateral to the PVN, tended to eat approximately 0.5 g more than those with cannulas aimed between A5340 μ and A4890 μ (immediately caudal to the PVN).

With regard to the stereotaxic coordinates for the placements, we have generally implanted the cannulas with

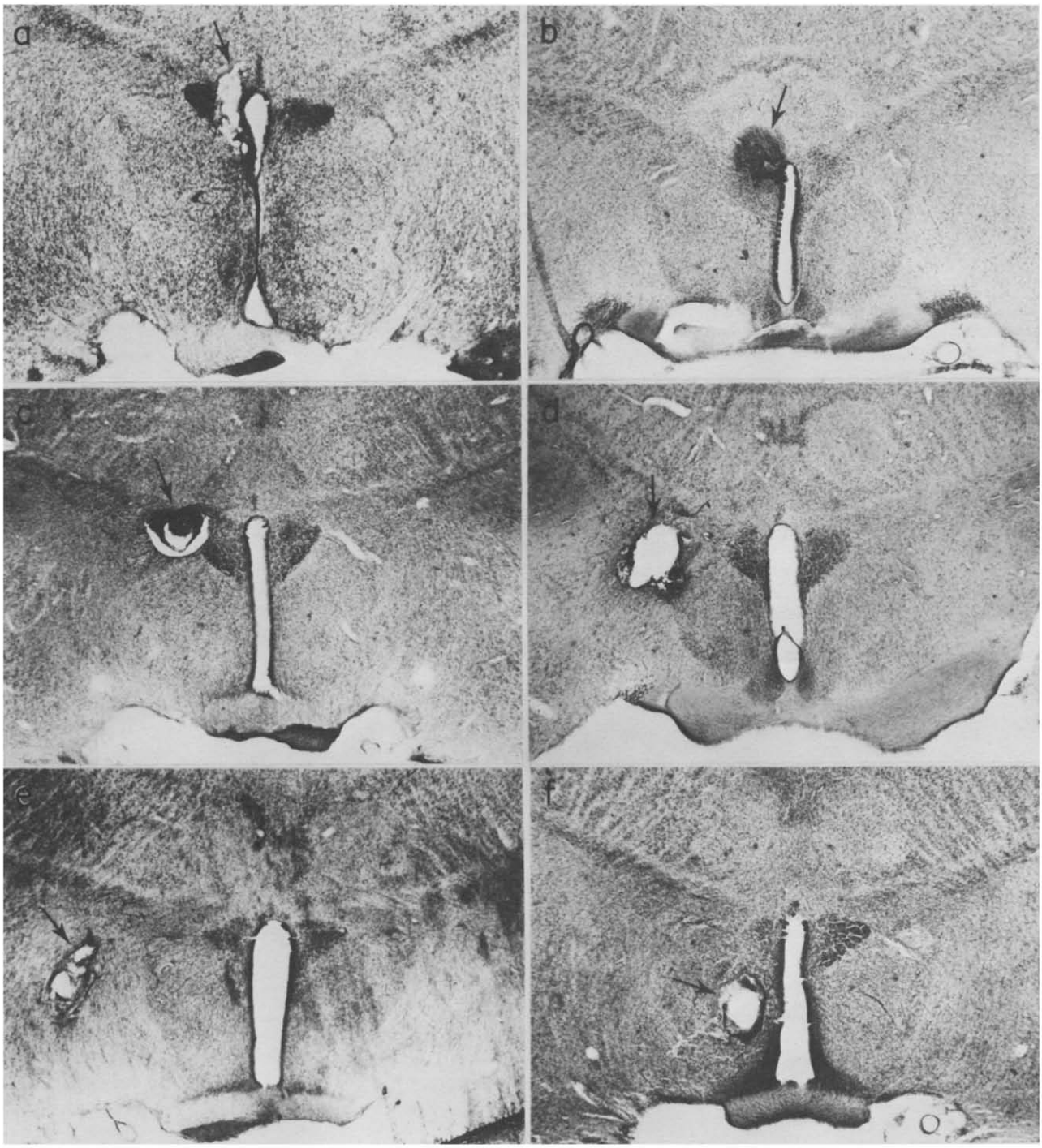

FIG. 3. Photomicrographs of frontal sections of the rat brain showing representative injection sites (indicated by arrows) for animals with unilateral cannulas aimed at: the paraventricular nucleus (a,b), just lateral (c) or ventral (f) to the nucleus, and at the fornix (d) or just lateral to the fornix in the lateral hypothalamus (e). See Fig. 2 for diagrammatic representation of these injection sites and and their respective behavioral sensitivity to norepinephrine.

the top of the incisor bar 3.1 mm above the center of the aural bars. Using this angle, the most effective coordinates for aiming a cannula directly at the dorsomedial portion of the PVN in adult male rats were as follows: 0.3 to 0.8 mm caudal to bregma, 0.2 to 0.4 mm lateral to the midline, and 8.1 to 8.3 mm ventral to the surface of the skull. (For routine testing, we frequently aim the cannula at the rostral tip of the PVN, that is, 0.0 to 0.3 mm caudal to bregma, to

minimize direct damage to the nucleus. We also use the more lateral placement (0.4 mm lateral) to avoid penetrating the saggital sinus). In a group of 15 rats, we attempted to convert these coordinates, for implantations to be performed with the skull flat. These preliminary trials with a change in head angle indicate the effective coordinates to be: 1.3 mm caudal to bregma, 0.3 mm lateral, and 7.8 mm ventral to skull surface. This placement, also aimed directly at the PVN, yielded a NE feeding response similar in magnitude to that obtained with the rat's head at an angle.

EXPERIMENT 3: MAPPING OF NE-ELICITED DRINKING

In addition to eliciting a feeding response, NE injected into the rostral hypothalamus has also been frequently found to elicit a small, rapid drinking response (generally between 1 and 4 ml) which occurs during the first few minutes prior to the initiation of eating [43]. This preprandial drinking can occur independently of the feeding response, that is, in the absence of food. However, it appears to be closely associated with the feeding process, since when food is present, it immediately precedes the onset of eating and the magnitude of each rat's drinking response is positively correlated with the magnitude of his feeding response [43].

In the present experiment, this preprandial drinking response was examined in a number of the rats used in Experiment 1 (on feeding), to determine whether the association, in magnitude and temporally, of these NE-elicited ingestive responses might actually reflect an anatomical contiguity of the mediating receptor systems. In this study, the rats (N = 75) were subjected to 4 additional tests with NE (40 nmoles) in which their latency to drink, as well as the amount of water drunk within 10 min after injection, was recorded. For these tests, only water was made available. As described previously [43], when food is also present, the drinking response is somewhat reduced in magnitude (that is, prematurely terminated), apparently due to the competitive stimulus to eat.

Results and Discussion

These results of these tests clearly indicate that the NE-elicited preprandial drinking response can be obtained only from sites in or near to the PVN. The anatomical mapping of sensitivity to NE reveals similar results for elicited feeding and drinking, with the magnitude of the drinking response diminishing more rapidly with increased distance from the PVN.

As with NE-induced feeding, essentially no drinking was observed after NE injection into extra-hypothalamic sites, nor into lateral or caudal hypothalamic sites. Within the medial, rostral portion of the hypothalamus, in contrast, NE induced a reliable drinking response at several sites, with the strongest effect (4.1 ml in approximately 3.7 min) occurring in the PVN. In Table 1, the effectiveness of this site can be seen relative to the reliable but smaller responses detected after injection into sites just anterior, lateral, or dorsal to the nucleus, and the statistically unreliable effects which occurred in the ventromedial and dorsomedial nuclei and with injection into the third ventricle. By comparing these drinking scores with the same rats' NE feeding scores obtained in Experiment 1 (also shown in Table 1), a quite

TABLE 1

NOREPINEPHRINE-ELICITED DRINKING AND FEEDING AS A FUNCTION OF HYPOTHALAMIC INJECTION SITE

Hypothalamic injection site	Relative to Paraventricular nucleus	N	Water and food intake after norepinephrine injection: Water (ml ± SEM)	Food (g ± SEM)
Paraventricular nucleus		12	4.1 ± 0.5‡	4.3 ±0.4‡
Rostral border of paraventricular nucleus	Anterior	14	3.2 ± 0.6‡	3.4 ± 0.4‡
Perifornical	Lateral	13	2.7 ± 0.7‡	3.3 ± 0.8‡
Nucleus Reuniens	Dorsal	9	1.8 ± 0.5†	2.4 ± 0.5†
Ventromedial nucleus	Ventral, and Posterior	10	1.0 ± 0.4	2.3 ± 0.6†
Third Ventricle	Dorsal, and Anterior	8	1.3 ± 0.7	2.1 ± 0.6†
Dorsomedial nucleus	Posterior	9	0.2 ± 0.1	1.8 ± 0.6*

Two-tailed dependent *t*-tests comparing saline (generally near zero) and norepinephrine scores.
*$p<0.05$.
†$p<0.01$.
‡$p<0.001$.

similar pattern of sensitivity can be seen. However, as is generally found to be the case, the feeding response appeared to be somewhat more robust than the drinking, as indicated by the reliable feeding that occurred at three medial sites where drinking could not be observed.

The food and water intake scores presented in Table 1 were found to be positively correlated ($r = +.96$, $p<0.001$) as a function of injection site. This anatomical association suggests that the noradrenergic receptor systems mediating these two ingestive behaviors may be contiguous, if not one and the same system. The dissociation of these two responses at the three sites where only feeding could be observed most probably reflects their differences in robustness or threshold sensitivity, which in turn may be due to differences in receptor density or sensitivity. As just mentioned above, the feeding response is more readily elicited than the drinking response at high NE doses, and, as revealed by tests with very low NE doses [43,49], the feeding can be initiated by lower threshold doses (approximately 4 ng) than the drinking (approximately 18 ng).

While the positive correlations between the two responses, both within a given animal [43] and as a function of injection site (Table 1), might reflect an anatomical as well as a functional link between them, there are two pieces of evidence which have dissociated these two phenomena both pharmacologically and hormonally. The pharmacological finding is that beta-adrenergic blockers are effective in antagonizing the drinking without affecting the feeding [44]. The hormonal dissociation was detected in hypophysectomized animals in which the eating was abolished while the drinking remained unaffected [52]. This dissociation of the two NE ingestive responses is interesting in light of the iontophoretic results of Moss *et al.* [61] who have distinguished two types of NE-sensitive units within the PVN. These investigators found NE to inhibit the firing of PVN neurons directly linked to the pituitary (as determined via antidromic stimulation) but to excite other neurons which appeared to be independent of the pituitary.

From these results, it seems that in the satiated animal, the PVN is NE's primary site of action for preprandial drinking behavior, as well as for eating behavior. Consistent with these mapping results are the scores obtained for the latency to respond after drug administration. When injected into the PVN, NE elicited a drinking response within approximately 0.8 min (ranging from 0.3 to 1.5 min) after injection. This relatively short latency contrasts with that observed after injection into sites just anterior or lateral to the PVN. At these sites, a somewhat weaker response was elicited (see Table 1) after a longer latency of 1.7 min (ranging from 0.9 to 2.6 min). These findings confirm our earlier results obtained with injection into the perifornical area of the anterior hypothalamus [43].

EXPERIMENT 4: NE EFFECTS ON FEEDING IN HUNGRY RATS

In addition to eliciting eating in satiated rats (as in Experiments 1 and 2), NE has been shown to potentiate the ongoing feeding response exhibited during a normal, spontaneously initiated meal [68] or as a result of deprivation-induced hunger [40,47]. The present series of tests in slightly food-deprived animals was designed to determine whether the NE enhancement of eating effect in already hungry rats is similar, in terms of its anatomical properties, to the NE-elicited feeding in satiated rats. Is the anteromedial hypothalamus, specifically the PVN, the site of greatest effect?

Four different anterior-posterior levels, extending from the preoptic area to the paraventricular and ventromedial nuclei and, finally, to the posterior hypothalamus, were examined (see Table 2). At each of these levels, unilateral cannulas were directed toward a medial site (0.3 mm lateral to the midline) or a lateral site (1.6 mm lateral to the midline). As determined histologically at the end of the experiment, the depth of these cannulas (see Table 2) generally placed the medial ones close to or within the structures indicated in the Table, and lateral cannulas (with the exception of the lateral preoptic area) alongside the fornix. All animals ($N = 74$; between 8 and 10 at each injection site) were tested with NE (40 nmoles) or saline, in counterbalanced order, after a 24-hr period of mild (20%) food deprivation. When food was made available to these animals, this deprivation schedule caused them, on saline tests, to initiate a discrete meal between 1.6 and 2.1 g in size and lasting between 10 and 15 min. Food intake was measured 30 min after drug injection and food presentation. Water was made available, but drinking results are not presented. A total of 8 tests, 4 with NE and 4 with saline, were conducted.

Results and Discussion

As can be seen in Table 2, the responsiveness to NE of slightly hungry rats exhibits an anatomical pattern similar to that observed in satiated animals. The PVN, where NE increased the meal size of hungry animals by 165%, was once again distinguished as the most sensitive region.

Two additional findings should be noted. First, the medial brain area sensitive to NE after mild food deprivation appeared to extend somewhat beyond that observed in satiated animals (Experiment 1). While the PVN was clearly the most effective site in both satiated and already hungry animals, the medial structures rostral and caudal to the PVN (those indicated in Table 2) appeared somewhat more sensitive in the hungry rats (see also [38]). For example, in these rats, NE significantly increased the size of an ongoing meal after injection medially into the rostral portion of the preoptic area or the posterior hypothalamus. In the satiated animal, however, no reliable effect of NE in initiating a meal was detected at these sites (Fig. 1). These results suggest that, due either to behavioral or to physiological factors, a lower threshold of sensitivity to NE may exist during the course of a meal, in comparison with preceding a meal.

The second point pertains to the effect of NE in the lateral hypothalamic regions. Our failure to obtain a stimulatory effect of NE in these areas of hungry rats (Table 2) confirms our results obtained in satiated rats (Fig. 1). In addition, however, we actually found NE in these lateral perifornical areas to cause the opposite effect, namely, a suppression of feeding. (In satiated rats, the low food intake baseline prevented this effect from being revealed.) This latter phenomenon appeared strongest at the level of the PVN and the ventromedial nucleus and, although small, reached statistical significance lateral to the PVN. This suppressive effect, which has been described previously (see [47]), is considerably more pronounced with the adrenergic agonist epinephrine, which appears to

TABLE 2

NOREPINEPHRINE IN MILDLY FOOD-DEPRIVED RATS: EFFECT ON MEAL SIZE AS A FUNCTION OF HYPOTHALAMIC INJECTION SITE

Identifying Structure	Anterior-Posterior Level*	Ventral Extent*	Percent Change in Meal Size† Medial Cannula (0.3mm)	Lateral Cannula (1.6 mm)
Preoptic area	A 6860 μ	-1.9	+ 42‡	-12
Paraventricular nucleus	A 5340 μ	-2.0	+165§	-29‡
Ventromedial nucleus	A 4380 μ	-3.1	+ 86‡	-25
Posterior hypothalamus	A 3750 μ	-2.9	+ 58‡	-17

*Based on König and Klippel's [35] atlas of the rat brain.

†Meal size after saline injection ranged from 1.6 to 2.1 g. Percent change was calculated from the ratio of amount eaten after norpeinephrine injection to amount eaten after saline. Norepinephrine and saline scores were each averaged over 4 tests given and then statistically compared by a two-tailed dependent t-test. There were between 8 and 10 rats with cannulas aimed at each injection site.

‡$p<0.05$.

§$p<0.001$.

exert its effect through a beta-adrenergic receptor [37,48], and in animals pretreated with a monoamine oxidase inhibitor [51]. It also occurs with dopamine via a dopaminergic receptor (see [48] for review), as well as with amphetamine, which acts through the release of endogenous catecholamines [46].

GENERAL DISCUSSION

Each of the four experiments just described clearly indicates that the PVN in the rostral hypothalamus is the most effective site for stimulating ingestive behavior with noradrenergic activation in the rat. The largest feeding and preprandial drinking responses were observed after NE injection into this nucleus of satiated rats; in slightly hungry rats, the strongest potentiation of an ongoing feeding response was also obtained at this site. The latency to respond was clearly shortest after NE stimulation of the PVN, and, as shown in some additional experiments with this agonist [49], this brain region has exhibited the lowest threshold of sensitivity in terms of the minimum drug dose (less than 4 ng for feeding and 18 ng for drinking) required to produce a reliable response.

The credibility of these mapping results, in terms of their implicating a discrete brain region in the mediation of a central drug effect, rests heavily on the question concerning the extent of NE spread from its injection site. There have been extensive discussions of this question in the literature (for review, see [62,70]), and, while a few points remain unresolved, it seems that when a relatively small injection volume is used (1.0 μl or less), a spread of approximately 1 mm in diameter can be expected to occur at the injection site, in addition to some drug spread up along the cannula shaft and into the ventricle.

In all of the studies published to date on this topic, compounds other than NE were tested. Since the pattern of spread may be expected to vary depending on the drug injected, as well as the site of injection, it seemed important for our purposes to examine specifically the spread that occurs with NE injection into behaviorally responsive hypothalamic areas. With this goal in mind, John L. Gerlach in the laboratory of Dr. N. E. Miller here at The Rockefeller University used the autoradiographic procedure, similar to that described by Gerlach and McEwen [17], to analyze the spread of ^{3}H–NE after injection into the perifornical hypothalamus along the lateral surface of the PVN. For these tests, NE was injected at a moderately high and behaviorally effective dose of 20 nmoles in a volume of 1 μl, and the animals were sacrificed a few minutes later at the onset of eating.

Preliminary results of this analysis can be found in Fig. 4, which illustrates two of the five brains analyzed to date. Within a week after cannula implantation (when the rats were generally sacrificed), a pocket of necrotic tissue is found to develop around the tip of the cannula. The diameter of this pocket (approximately 0.8 mm) is slightly larger than the outer diameter of the 23-gauge cannula (0.64 mm). After injection of ^{3}H–NE, a highly concentrated area of radioactivity can be seen at the cannula tip, remaining to a large degree confined within the necrotic area, although spreading slightly beyond the perimeter by 0.1 to 0.2 mm. Thus, the total spread at the tip of the cannula (after a 1-μl injection) appears to be in the order of approximately 1 mm. This finding is supportive of our behavioral results demonstrating clear differences in responsiveness with 1-mm shifts in the injection site. With smaller cannulas and injection volumes, greater sensitivity in terms of diffentiating brain areas less than 1 mm apart may possibly be achieved. Such was apparently the case in Experiment 2 in which small but detectable behavioral differences were observed with approximately 0.5-mm changes in cannula placement relative to the center of the PVN.

In Fig. 4, some additional spread of NE can be seen along the outer surface of the cannula shaft, subsequently into the ventricles through which the cannula generally passes. The ingestive responses elicited by NE injection into the PVN, however, do not appear to be attributed to this

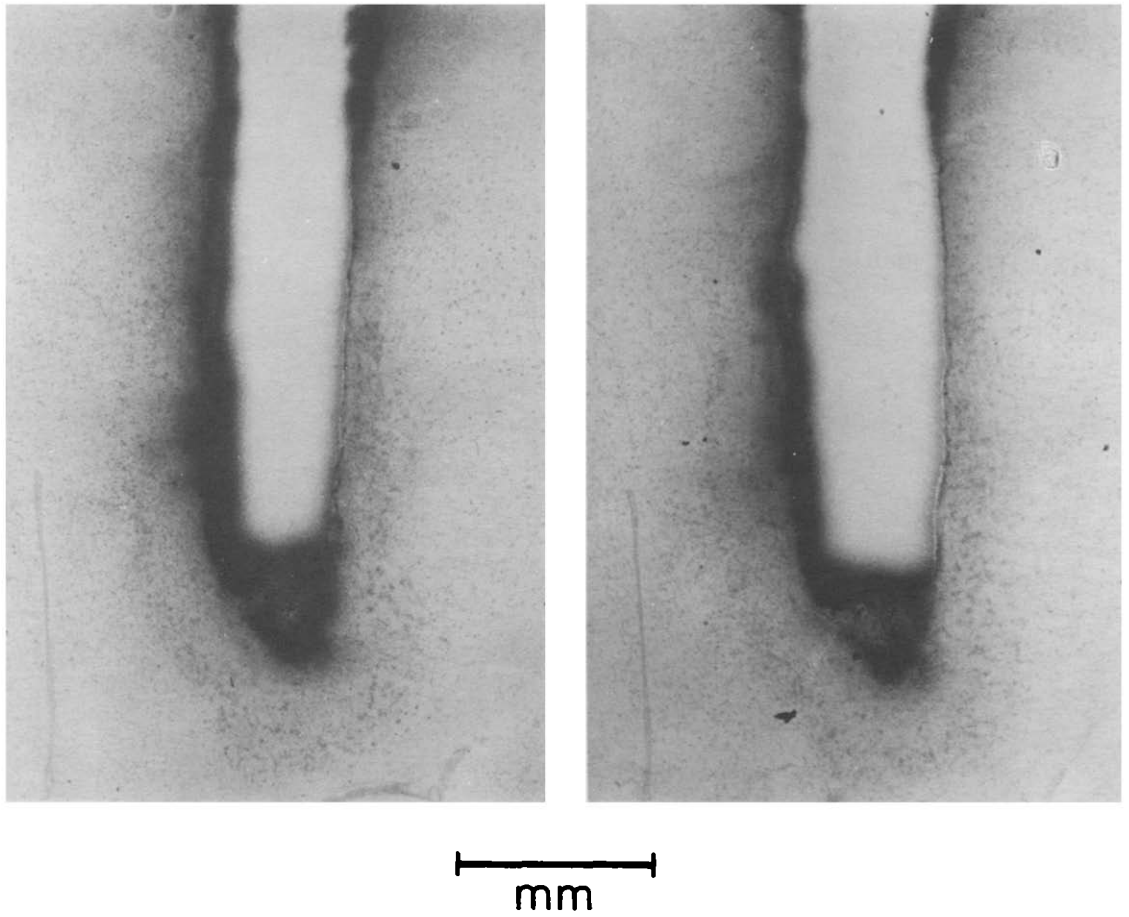

FIG. 4. Autoradiograms of ^{3}H-norepinephrine after injection into the perifornical hypothalamus, at the level of the paraventricular nucleus (see Fig. 3d), through chronically implanted brain cannulas in two rats. See Gerlach and McEwen [17] for details of autoradiographic procedure and text for experimental protocol.

route of spread. While NE injected directly into the lateral or third ventricles elicits reliable eating (see Fig. 1 and [2,3]), this response is considerably smaller than that observed with direct PVN injection; it occurs after a longer latency; and it is not associated with the PVN preprandial drinking response (Table 1). Furthermore, dramatic changes in sensitivity to NE (see Figs. 1 and 2 and Table 1), or even reversal of its predominant effect to a feeding suppression (Table 2), can be observed with cannula shifts that maintain the position of the cannula relative to the third or lateral ventricles but increase its distance relative to the PVN. The reduced sensitivity of sites near to the PVN, in particular the perifornical, ventromedial, and dorsomedial hypothalamic areas, may reflect either a reduced number of receptors situated at these sites outside the PVN or the spread of NE from these areas to the PVN. While the available evidence does not permit a definitive choice between these alternatives, the latter possibility seems most likely since cannulas aimed at the ventromedial area generally pass through or near to the PVN; the perifornical and dorsomedial areas lie only 0.5 mm or so from the PVN; and, most importantly, injection sites just lateral to these structures which border the PVN (Figs. 1 and 2) were relatively unresponsive to noradrenergic stimulation. The small feeding response elicited in the hippocampus or the thalamic periventricular area may also be due to drug spread to the PVN, in this case very probably via the ventricles.

In addition to initiating feeding in satiated rats, NE in the mildly hungry rat was found to increase the strength of an ongoing feeding response. Our mapping analysis of this phenomenon indicated its focal point of sensitivity to be the PVN, just as with NE-elicited feeding. In hungry animals, however, we were able to detect a somewhat wider area of NE receptivity, extending rostral and caudal along the medial hypothalamus into sites where NE generally failed to initiate feeding in satiated rats. This finding is consistent with the results of a recent study [68] in which NE was shown to be more effective (that is, effective at a lower threshold dose) in potentiating a normal, spontaneously initiated meal than in eliciting a new meal in a nonfeeding animal. Thus, in both this and the present study, normal or deprivation-induced hunger was found to be associated with an increase in responsiveness to NE. While this enhancement may simply reflect a greater behavioral receptivity in animals already in the process of feeding, it may also reveal a physiological change in the brain, perhaps at the synaptic level, which develops with

increased hunger. This change may involve an increase in release of endogenous NE (such as in [59]) or an increase in receptor sensitivity or number (such as in [69]). Both changes would be expected to potentiate the action of exogenous NE, and this increased effectiveness may be revealed either by a lower threshold dose or by a wider brain area over which NE may effectively diffuse. In the present study in hungry rats, the wider area of NE receptivity, extending beyond the PVN along the medial hypothalamus, may simply reflect an increase in receptor sensitivity within the PVN itself. This receptor change would yield a greater response of the nucleus to lower concentrations of NE spreading from more distant injection sites. An additional possibility, however, is that with increased levels of hunger a wider range of adrenergically innervated brain tissue may actually become more actively involved. Such a recruitment may help to initiate the necessary regulatory responses for restoring normal energy balance.

Thus, from the present study, it still remains an open question whether NE stimulation of feeding involves predominantly the PVN, or whether a more extended medial area, with the PVN as its focus, also plays a role. Histochemical or biochemical assays of catecholamine neurons in the hypothalamus (NE and epinephrine) have revealed a particularly rich innervation of the PVN as well as the periventricular regions of the hypothalamus [32, 33, 53–55, 63, 64, 77, 78]. It is possible therefore that a more extended area within the periventricular zone may become involved with increased levels of hunger. In light of these findings, it is important to mention the recent studies of Martin and Myers [59] in which the push-pull cannula technique was used to measure the efflux of ^{14}C-labeled endogenous NE during a normal meal in a hungry animal. These authors found that, in the feeding rat, a reliable increase in the efflux of ^{14}C-NE occurred in the anterior portion of the third ventricle, at the level of the paraventricular nucleus as well as caudal towards the ventromedial nucleus (Myers, personal communication). No change in NE was observed in the lateral ventricle, nor in the region of the anterior hypothalamus. These findings, together with the results of our mapping studies, converge on the medial PVN region as being a key link in the process of increased noradrenergic activity associated with increased food consumption in the rat. This relationship may be reflected in the finding of Cruce *et al.* [13] that genetically obese Zucker rats have decreased NE content specifically in the PVN, possibly as a result of increased NE release associated with insufficient acceleration of synthesis.

Further evidence which points toward the rostral hypothalamus, perhaps the PVN, as being crucial in the regulation of feeding are the results obtained with knife cuts and lesions. Lesions in the area of the ventromedial nucleus are known to produce overeating and obesity (see [26]). This effect, however, is believed not to involve the nucleus itself but to be due to tissue damage rostral (in the anterior hypothalamus) and perhaps immediately lateral to the nucleus [19]. The relationship of this effect to the PVN noradrenergic mechanism for stimulating feeding is not clear at this time, although it has been demonstrated that feeding elicited by NE injection into the perifornical region can be abolished by ventromedial hypothalamic lesions [29]. In this study, not all ventromedial lesions were effective in antagonizing the NE response, nor was there a clear relationship between NE blockade and the development of obesity, which typically results from this lesion. In light of our evidence pointing toward a PVN or periventricular location of the crucial adrenergic receptors, the disruption of NE's feeding effect after ventromedial hypothalamic lesions most probably does not reflect direct damage to these receptors.

Alternative explanations of this phenomenon may be found in the hormonal consequences of the lesion (that is, disruption of pituitary function), in the possible destruction of fibers of passage, and/or the damage to specific PVN afferents or efferents linking the brain and the peripheral autonomic nervous system [16, 20, 26, 67]. Feeding elicited by hypothalamic injection of NE has been found to be dramatically altered by changes in circulating adrenal hormones [50,52]. Furthermore, changes in autonomic function has been observed after NE injection [8,75], and the elicited feeding response is found to be abolished by surgical and pharmacological blockade of the vagus nerve [Leibowitz, unpublished observations]. It is interesting to note that while ventromedial lesions in the Herberg and Franklin study were effective in antagonizing the NE-elicited feeding response, lesions placed just lateral to these, in the lateral hypothalamic region, appeared to have little effect on the response [3]. In this latter study, NE was injected into the ventricles, and the elicited adrenergic feeding response remained intact even in some rats which were generally aphagic as a result of the lesion. This evidence is consistent with our findings that NE has little stimulatory action (or even produces a suppressive effect) after injection directly into the lateral hypothalamus (Fig. 1 and [40]).

In addition to the above effect observed with medial hypothalamic lesions, parasagittal knife cuts aimed between the medial and lateral hypothalamic regions have also been found to produce overeating [26]. Interestingly, as with the NE- and lesion-induced eating phenomena, the most effective site for increasing food intake with parasagittal cuts appears to be at the level of the PVN [18, 66, 72]. Coronal knife cuts also produce hyperphagia and increase weight gain [1, 25, 73] and this effect, which can be observed with cuts in the midbrain as well as throughout the hypothalamus, disappears immediately rostral to the PVN [20].

These results reveal the coronal level of the PVN as the rostral focus of a longitudinal pathway for inhibiting food intake. The relationship between this system and that mediating NE-elicited feeding is not clear at this time. It has been proposed that hypothalamic injection of NE may stimulate food intake via disinhibition; that is, by inhibition of satiety [10, 38, 39, 68]. Evidence to support this view may be obtained from iontophoretic studies that have demonstrated a predominantly inhibitory effect of NE on neuronal discharge in the region of the PVN [4, 12, 61].

What then is the relationship between NE stimulation of feeding with PVN injection and hyperphagia resulting from knife cuts near to the nucleus? If NE acts in an inhibitory fashion to suppress fibers of satiety, overeating and obesity after knife cuts could not be attributed to the severing of noradrenergic input to the PVN. Such an effect would be expected to produce the opposite outcome, namely, increased satiety and loss of body weight. With regard to the possibility that the knife cuts damage the efferents of a PVN noradrenergic feeding system, the autoradiographic evidence of Conrad and Pfaff [9] bears directly on this

issue. These authors indicate that the PVN, in addition to projecting rostrally and to the supraoptic nucleus and median eminence, sends a band of fibers caudally along the ventral surface of the hypothalamus (partially within the supraoptic commissure of Meyner), in the periventricular hypothalamus adjacent to the third ventricle, and a few axons laterally from the PVN, through the lateral hypothalamus into the anterior and medial amygdaloid nuclei. In mapping studies on hyperphagia caused by coronal knife cuts, it has been shown that damage limited to the midline has little effect on feeding and that mid-lateral cuts need not extend medially to produce a reliable effect [20, 27, 65, 66, 72]. In a study with short parasagittal cuts at the level of the PVN [34], hyperphagia has been found to occur with a dorsal cut just lateral to the PVN and to a somewhat greater extent with a short cut along the ventral surface. The anatomical overlap of these knife-cut results and of Conrad and Pfaff's autoradiographic analysis does not provide any clear indication as to which PVN efferents (if any) may be involved in the hyperphagia caused by the knife cuts. Tentatively, it may be said that midline projections are not crucial, whereas ventral and lateral projections might be contributing to, but not essential for, the phenomenon. Other neurochemical systems are undoubtedly involved in the hyperphagia and obesity after hypothalamic knife cuts, and possible candidates might be the ascending catecholaminergic projection in the perifornical region (for review, see [47,48]) and also an ascending serotonergic fiber system which, if ablated, produces hyperphagia and increased growth (see [31,73]).

The evidence just summarized distinguishes the coronal level of the PVN as an important area for control of food ingestion. Until recently, this area has remained largely unexplored, since most earlier studies have focused their attention on more caudal (ventromedial, dorsomedial, and lateral) sites in the hypothalamus. The PVN has generally not been associated with feeding behavior, although in an early study lesions in this nucleus were suggested to produce hyperphagia in the dog [28]. Furthermore, ventral hypothalamic lesions that included, or extended immediately ventral to, the PVN were found to be particularly effective in producing hyperphagia in the rat [19]. Additional studies with discrete PVN lesions are clearly required to define more precisely the role of this nucleus in control of feeding.

Most commonly, the PVN has been associated with the control of hormone release by the pituitary (e.g. [7, 12, 58, 71]), and the rich catecholamine innervation of this region is believed to play an important role in this neuroendocrine process [79]. To gain insight into a possible link between the noradrenergic feeding effects involving the PVN and its normal neuroendocrine function, this laboratory has examined the effect of a variety of hormone manipulations on the phenomenon of NE-elicited feeding [50,52]. The outcome of these studies has demonstrated a clear dependence of this NE response on circulating adrenal hormones. Adrenalectomy and hypophysectomy are found to abolish the NE feeding effect, and replacement with corticosterone or, to some extent, desoxycorticosterone is found to restore the response. This relationship, although a permissive one, may in some manner reflect the function of the PVN relative to adrenocorticotrophin release from the pituitary [15]. It may also be related to the finding of other studies [36,60] which have associated normal feeding periods with circadian rises in endogenous corticosterone.

REFERENCES

1. Albert, D. J., L. H. Storlien, J. G. Albert and C. J. Mah. Obesity following disturbance of the ventromedial hypothalamus: A comparison of lesions, lateral cuts, and anterior cuts. *Physiol. Behav.* 7: 135–141, 1971.
2. Antunes-Rodrigues, J. and S. M. McCann. Water, sodium chloride, and food intake induced by injections of cholinergic and adrenergic drugs into the third ventricle of the rat brain. *Proc. Soc. exp. Biol. Med.* 133: 1464–1470, 1970.
3. Berger, B. D., C. D. Wise and L. Stein. Norepinephrine: Reversal of anorexia in rats with lateral hypothalamic damage. *Science* 172: 281–284, 1971.
4. Bloom, F. E., A. P. Oliver and G. C. Salmoiraghi. The responsiveness of individual hypothalamic neurons to microelectrophoretically administered endogenous amines. *Int. J. Neuropharmac.* 2: 181–193, 1963.
5. Booth, D. A. Localization of the adrenergic feeding system in the rat diencephalon. *Science* 158: 515–517, 1967.
6. Booth, D. A. Mechanism of action of norepinephrine in eliciting an eating response on injection into the rat hypothalamus. *J. Pharmac. exp. Ther.* 160: 336–348, 1968.
7. Brownstein, M. J., M. Palkovits, J. M. Saavedra, R. M. Bassiri and R. D. Utiger. Thyrotropin-releasing hormone in specific nuclei of rat brain. *Science* 185: 267–268, 1974.
8. Carmona, A. and J. L. Slangen. Effects of chemical stimulation of the hypothalamus upon gastric secretion. *Physiol. Behav.* 10: 657–661, 1973.
9. Conrad, L. C. A. and D. W. Pfaff. Efferents from medial basal forebrain and hypothalamus in the rat: An autoradiographic study of the anterior hypothalamus. *J. comp. Neurol.* 169: 221–261, 1976.
10. Coons, E. E. and D. Quartermain. Motivational depression associated with norepinephrine-induced eating from hypothalamus: Resemblance to the ventromedial hyperphagic syndrome. *Physiol. Behav.* 5: 687–692, 1970.
11. Coury, J. N. Neural correlates of food and water intake in the rat. *Science* 156: 1763–1765, 1967.
12. Cross, B. A., R. E. J. Dyball, R. G. Dyer, C. W. Jones, D. W. Lincoln, J. F. Morris and B. T. Pickering. Endocrine neurons. In: *Recent Progress in Hormone Research*, Vol. 31, edited by R. O. Greep. New York: Academic press, 1975, pp. 243–294.
13. Cruce, J. A. F., N. B. Thoa and D. M. Jacobowitz. Catecholamines in the brains of genetically obese rats. *Brain Res.* 101: 165–170, 1976.
14. Davis, J. R. and R. E. Keesey. Norepinephrine-induced eating – Its hypothalamic locus and an alternative interpretation of action. *J. comp. physiol. Psychol.* 77: 394–402, 1971.
15. Defendini, R. and E. A. Zimmerman. The magnocellular neurosecretory system of the mammalian hypothalamus. In: *Res. Publ. ARNMD*, Vol. 56, 1977, in press.
16. Friedman, M. I. and E. M. Stricker. The physiological psychology of hunger: A physiological perspective. *Psychol. Rev.* 83: 409–431, 1976.
17. Gerlach, J. L. and B. S. McEwen. Rat brain binds adrenal steroid hormone: Radioautography of hippocampus with corticosterone. *Science* 175: 1133–1136, 1972.
18. Gold, R. M. Hypothalamic hyperphagia produced by parasagittal knife cuts. *Physiol. Behav.* 5: 23–25, 1970.
19. Gold, R. M. Hypothalamic obesity: The myth of the ventromedial nucleus. *Science* 182: 488–490, 1973.

20. Gold, R. M., A. P. Jones, P. E. Sawchanko and G. Kapatos. Paraventricular area: Critical focus of a longitudinal neurocircuitry mediating food intake. *Physiol. Behav.*, 1977, in press.
21. Goldman, H. W., D. Lehr and E. Friedman. Antagonistic effects of alpha- and beta-adrenergically coded hypothalamic neurones on consummatory behaviour in the rat. *Nature, Lond.* **231**: 453–455, 1971.
22. Grossman, S. P. Direct adrenergic and cholinergic stimulation of hypothalamic mechanisms. *Am. J. Physiol.* **202**: 872–882, 1962.
23. Grossman, S. P. Effects of adrenergic and cholinergic blocking agents on hypothalamic mechanisms. *Am. J. Physiol.* **202**: 1230–1236, 1962.
24. Grossman, S. P. Behavioral effects of chemical stimulation of the ventral amygdala. *J. comp. physiol. Psychol.* **57**: 29–36, 1964.
25. Grossman, S. P. Changes in food and water intake associated with an interruption of the anterior or posterior fiber connections of the hypothalamus. *J. comp. physiol. Psychol.* **75**: 23–31, 1971.
26. Grossman, S. P. Role of the hypothalamus in the regulation of food and water intake. *Psychol. Rev.* **82**: 200–224, 1975.
27. Grossman, S. P. and J. W. Hennessy. Differential effects of cuts through the posterior hypothalamus on food intake and body weight in male and female rats. *Physiol. Behav.* **17**: 89–102, 1976.
28. Heinbecker, P., H. L. White and D. Rolf. Experimental obesity in the dog. *Am. J. Physiol.* **141**: 549–565, 1944.
29. Herberg, L. J. and K. B. K. Franklin. Adrenergic feeding: Its blockade or reversal by posterior VMH lesions; and a new hypothesis. *Physiol. Behav.* **8**: 1029–1034, 1972.
30. Hoebel, B. G. Feeding: Neural control of intake. *Ann. Rev. Physiol.* **33**: 533–568, 1971.
31. Hoebel, B. G. The pharmacology of feeding. In: *Handbook of Psychopharmacology*, edited by L. L. Iversen, S. D. Iversen and S. H. Snyder. New York: Plenum, 1977, in press.
32. Hökfelt, T., K. Fuxe, M. Goldstein and O. Johansson. Immunohistochemical evidence for the existence of adrenaline neurons in the rat brain. *Brain Res.* **66**: 235–251, 1974.
33. Jacobowitz, D. M. and M. Palkovits. Topographic atlas of catecholamine and acetylcholinesterase-containing neurons in the rat brain. I. Forebrain (telencephalon, diencephalon). *J. comp. Neurol.* **157**: 13–28, 1974.
34. Jones, A. P., S. D'Angelo and P. Sawchanko. Localization of satiety neurocircuitry utilizing asymmetrical knife cuts. Paper presented at 47th Annual Meeting of Eastern Psychological Association, 1976.
35. König, J. F. R. and R. A. Klippel. *The Rat Brain: A Stereotaxic Atlas.* Baltimore: Williams and Wilkins, 1963.
36. Krieger, D. T. Food and water restriction shifts corticosterone, temperature, activity and brain amine periodicity. *Endocrinology* **95**: 1195–1201, 1974.
37. Leibowitz, S. F. Hypothalamic β-adrenergic "satiety" system antagonizes an α-adrenergic "hunger" system in the rat. *Nature, Lond.* **226**: 963–964, 1970.
38. Leibowitz, S. F. Reciprocal hunger-regulating circuits involving alpha- and beta-adrenergic receptors located, respectively, in the ventromedial and lateral hypothalamus. *Proc. natn. Acad. Sci. U.S.A.* **67**: 1063–1070, 1970.
39. Leibowitz, S. F. Central adrenergic receptors and the regulation of hunger and thirst. In: *Neurotransmitters*, edited by I. J. Kopin. *Res. Publ. ARNMD*, Vol. 50, 1972, pp. 327–358.
40. Leibowitz, S. F. Brain norepinephrine and ingestive behaviour. In: *Frontiers in Catecholamine Research*, edited by E. Usdin and S. H. Snyder. Oxford: Pergamon press, 1973, pp. 711–713.
41. Leibowitz, S. F. Adrenergic receptor mechanisms in eating and drinking. In: *The Neurosciences, Third Study Program*, edited by F. O. Schmitt and F. G. Worden. Cambridge: MIT Press, 1974, pp. 713–719.
42. Leibowitz, S. F. Paraventricular nucleus: A primary site mediating adrenergic feeding-elicitation. Paper presented at meeting of Eastern Psychological Association, Philadelphia, 1974.
43. Leibowitz, S. F. Pattern of drinking and feeding produced by hypothalamic norepinephrine injection in the satiated rat. *Phsyiol. Behav.* **14**: 731–742, 1975.
44. Leibowitz, S. F. Ingestion in the satiated rat: Role of alpha and beta receptors in mediating effects of hypothalamic adrenergic stimulation. *Physiol. Behav.* **14**: 743–754, 1975.
45. Leibowitz, S. F. Amphetamine: Possible site and mode of action for producing anorexia in the rat. *Brain Res.* **84**: 160–167, 1975.
46. Leibowitz, S. F. Catecholaminergic mechanisms of the lateral hypothalamus: Their role in the mediation of amphetamine anorexia. *Brain Res.* **98**: 529–545, 1975.
47. Leibowitz, S. F. Brain catecholaminergic mechanisms for control of hunger. In: *Hunger: Basic Mechanisms and Clinical Implications*, edited by D. Novin, W. Wyrwicka and G. Bray. New York: Raven Press, 1976, pp. 1–18.
48. Leibowitz, S. F. Catecholaminergic mechanisms of the lateral hypothalamus: Their role in the mediation of amphetamine anorexia. In: *Central Mechanisms of Anorectic Drugs*, edited by S. Garattini and R. Samanin. New York: Raven Press, 1978, in press.
49. Leibowitz, S. F. Adrenergic stimulation of the paraventricular nucleus: Elicited feeding as a function of dose and time of injection in day-night cycle. Submitted manuscript, 1977.
50. Leibowitz, S. F. Feeding elicited by noradrenergic stimulation of the paraventricular nucleus: Meal size as related to varying plasma corticosterone levels. Paper presented at VIth International Conference on the Physiology of Food and Fluid Intake, Paris, 1977.
51. Leibowitz, S. F. and C. Rossakis. Catecholamines of the perifornical lateral hypothalamic region exert an inhibitory influence on feeding behavior in the rat. Paper presented at Meeting of Eastern Psychological Association, New York City, 1976.
52. Leibowitz, S. F., K. Chang and R. L. Oppenheimer. Feeding elicited by noradrenergic stimulation of the paraventricular nucleus: Effects of corticosterone and other hormone manipulations. *Neurosci. Abstr.* **2**: 292, 1976.
53. Lindvall, O. and A. Björklund. The organization of the ascending catecholamine neuron systems in the rat brain as revealed by the glyoxylic acid fluorescence method. *Acta physiol. scand.* Suppl. 412: 1–48, 1974.
54. Lindvall, O., A. Björklund, A. Nobin and U. Stenevi. The adrenergic innervation of the rat thalamus as revealed by the glyoxylic acid fluorescence method. *J. comp. Neurol.* **154**: 317–348, 1974.
55. Maeda, T. and N. Shimizu. Projections ascendantes du locus coeruleus et d'autres neurones aminergiques pontiques au niveau du presencéphale du rat. *Brain Res.* **36**: 19–35, 1972.
56. Margules, D. L. Alpha-adrenergic receptors in hypothalamus for the suppression of feeding behavior by satiety. *J. comp. physiol. Psychol.* **73**: 1–12, 1970.
57. Margules, D. L. Beta-adrenergic receptors in the hypothalamus for learned and unlearned taste aversions. *J. comp. physiol. Psychol.* **73**: 13–21, 1970.
58. Martin, J. B., R. Boshaus and S. Reichlin. Feedback regulation of TSH secretion in rats with hypothalamic lesions. *Endocrinology* **87**: 1032–1040, 1970.
59. Martin, G. E. and R. D. Myers. Evoked release of [^{14}C]-norepinephrine from the rat hypothalamus during feeding. *Am. J. Physiol.* **229**: 1547–1555, 1975.
60. Moberg, G. P., L. L. Bellinger and V. E. Mendel. Effect of meal feeding on daily rhythms of plasma corticosterone and growth hormone in the rat. *Neuroendocrinology* **19**: 160–169, 1975.
61. Moss, R. L., I. Urban and B. A. Cross. Microelectrophoresis of cholinergic and aminergic drugs on paraventricular neurons. *Am. J. Physiol.* **223**: 310–318, 1972.

62. Myers, R. D. *Handbook of Drug and Chemical Stimulation of the Brain.* New York: Van Nostrand Reinhold, 1974.
63. Olson, L. and K. Fuxe. Further mapping out of central noradrenaline neuron systems: Projections of the "subcoeruleus" area. *Brain Res.* 43: 289–295, 1972.
64. Palkovits, M., M. Brownstein, J. M. Saavedra and J. Axelrod. Norepinephrine and dopamine content of hypothalamic nuclei of the rat. *Brain Res.* 77: 137–149, 1974.
65. Paxinos, G. and D. Bindra. Hypothalamic knife cuts: Effects on eating, drinking, irritability, aggression, and copulation in the male rat. *J. comp. physiol. Psychol.* 79: 219–229, 1972.
66. Paxinos, G. and D. Bindra. Hypothalamic and midbrain neural pathways involved in eating, drinking, irritability, aggression, and copulation. *J. comp. physiol. Psychol.* 82: 1–14, 1973.
67. Powley, T. L. The ventromedial hypothalamic syndrome, satiety, and a cephalic phase hypothesis. *Psychol. Rev.* 84: 89–126, 1977.
68. Ritter, R. C. and A. N. Epstein. Control of meal size by central noradrenergic action. *Proc. natn. Acad. Sci. U.S.A.* 72: 3740–3743, 1975.
69. Romero, J. A., M. Katz, J. W. Kebabian and J. Axelrod. Circadian cycles in binding of ^{3}H–alprenolol to β-adrenergic receptor sites in rat pineal. *Nature, Lond.* 258: 435–436, 1975.
70. Routtenberg, A. Intracranial chemical injection and behavior: A critical review. *Behav. Biol.* 7: 601–641, 1972.
71. Sawano, S., A. Arimura, A. V. Schally, T. Saito, C. Y. Bowers, C. P. O'Brien and L. M. N. Bach. Growth hormone-releasing activity in the hypothalami of kittens with lesions of the region of the paraventricular nuclei. *Acta endocr.* 59: 317–324, 1968.
72. Sclafani, A. Neural pathways involved in the ventromedial hypothalamic lesion syndrome in the rat. *J. comp. physiol. Psychol.* 77: 70–96, 1971.
73. Sclafani, A. and C. N. Berner. Hyperphagia and obesity produced by parasagittal and coronal hypothalamic knife cuts: Further evidence for a longitudinal feeding inhibitory pathway. *J. comp. physiol. Psychol.* 91: 1000–1018, 1977.
74. Slangen, J. L. and N. E. Miller. Pharmacological tests for the function of hypothalamic norepinephrine in eating behavior. *Physiol. Behav.* 4: 543–552, 1969.
75. Steffens, A. B. and J. H. Strubbe. Blood composition and feeding behaviour. Paper presented at Vth International Conference on the Physiology of Food and Fluid Intake, Jerusalem, 1974.
76. Storlien, L. H. and D. J. Albert. The effect of VMH lesions, lateral cuts and anterior cuts on food intake, activity level, food motivation and reactivity to taste. *Physiol. Behav.* 9: 191–197, 1972.
77. Ungerstedt, U. Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta physiol. scand.* Suppl. 367: 1–48, 1971.
78. Versteeg, D. H. G., J. van der Gugten, W. de Jong and M. Palkovits. Regional concentrations of noradrenaline and dopamine in rat brain. *Brain Res.* 113: 563–574, 1976.
79. Wilson, C. A. Hypothalamic amines and the release of gonadotrophins and other anterior pituitary hormones. *Adv. Drug Res.* 8: 119–204, 1974.