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Is the Noradrenergic "Feeding Circuit" in Hypothalamus Really an Olfactory System?

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MYERS, R. D., M. L MCCALEB AND K. A. HUGHES. *Is the noradrenergic "feeding circuit" in hypothalamus really an olfactory system?* PHARMAC. BIOCHEM BEHAV. 10(6) 923–927, 1979.—Since the act of feeding releases catecholamines from certain areas of the rat's hypothalamus, we have examined the possibility of a sensory component underlying this release. An individual diencephalic site in the unanesthetized rat was radiolabeled by microinjection of 1.0–2.0 μCi of ^{14}C-NE, in a volume of 0.5–1.0 μl, through a permanently implanted guide cannula. Then 30 min later, the labeled site was perfused by means of push-pull cannulae, with an osmotically balanced CSF solution at a rate of 25 μl/min. The interval of perfusion was 5 min with 10 min intervening. In the midpoint of a sequence of 7 perfusions, either the rat was allowed to consume food, or one of two odoriferous substances was placed beneath its cage floor, i.e., peanut butter or pyridine. During the presentation of either of the two olfactory stimuli to the animal, the efflux of ^{14}C-NE was enhanced from the same circumscribed site of perfusion in which feeding augmented similarly the release of the ^{14}C-NE isotope. An analysis of selected hypothalamic perfusates by TLC verified quantitatively the alteration in the profile of ^{14}C-NE and its labeled metabolites. Our results thus support the view that the changes in the catecholamine release within the hypothalamus reflect synaptic activity of the sensory pathways which mediate the olfactory input to this diencephalic structure.

Noradrenergic activity Feeding Hypothalamus Olfactory stimulation Push-pull perfusion

RECENTLY it was shown that as a rat eats food pellets, norepinephrine (NE) and its O-methylated metabolites are actively released within the hypothalamus but only from circumscribed sites [10]. This finding has provided direct physiological evidence for the existence of a noradrenergic feeding system in this diencephalic structure [16]. Subsequent experiments have revealed that a direct elevation in the local hypothalamic content of a nutrient such as glucose likewise causes a marked change in the pattern of NE release [11].

During the course of our studies on the activity of this catecholamine within the diencephalon, new evidence for a sensory component related to amine release has been uncovered. We observed that the hypothalamic release of NE suddenly increases in the experimental animal when another test rat or a person consumes food in its presence [12]. In the present investigation, therefore, the kinetics of the in vivo release of ^{14}C-NE and metabolites were examined under three conditions: feeding, exposure to peanut butter odor or to pyridine odor. The purpose of these procedures was to characterize the nature of the amine's activity while the animal sniffed a highly distinctive odor possessing either food or non-food properties.

METHOD

Male rats of the Long-Evans and Sprague-Dawley strains were maintained on a 12-hour dark, 12-hour light cycle. Push-pull guide tubes were cut from 20 ga thin-wall stainless steel tubing to the length of 15 mm and implanted stereotaxically [13] in fourteen rats under sodium pentobarbital anesthesia (35 mg/kg), according to the following set of coordinates [7]: AP= +5.0 to +7.5: Lat= +0.5 to +1.5: Hor= −6.0 below dura. The tip of each beveled guide tube was positioned 1.0 to 3.0 mm dorsal to the intended site of perfusion.

Post-operatively, 2–5 days later, the animal was fasted for 18 hr in order to maximize its response to olfactory stimulation. Then the site to be perfused was labeled with 1.0–2.0 μCi of DL(methylene-^{14}C)NE bitartrate (specific activity=53 mCi/mmol) (Amersham/Searle) microinjected in a volume of 0.5–1.0 μl over 10–20 sec. After 30 min, the NE labeled site was perfused with an artificial CSF [13] at a rate of 25 μl per min. The perfusion solution was acidified to pH 3.8 with 0.1 mg/ml of ascorbic acid and passed through an 0.22 μm Swinnex millipore filter. Each perfusion in a series lasted for 5 min with a 10 min interval intervening between successive perfusions during which time the Harvard "push-pull" pump was stopped [14].

In the midpoint of the perfusion series, usually during the fourth perfusion (i.e., at the 45 min interval) when the level of radioactivity in successive perfusates had stabilized, one of two experimental conditions was introduced: (a) the animal was permitted to consume 45 mg Noyes food pellets during the 5 min perfusion interval; or (b) one of two intense odorants was placed 3.0 cm beneath the cage floor of the

TABLE 1

CONCORDANCE OF MAXIMAL NE EFFLUX OF ^{14}C RADIOACTIVITY FROM HYPOTHALAMIC OR ADJACENT SITES IN INDIVIDUAL RATS THE THREE EXPERIMENTAL CONDITIONS WERE USUALLY TESTED AT 24 HR INTERVALS (1) FEEDING (2) EXPOSURE TO PEANUT BUTTER ODOR (PB). (3) EXPOSURE TO PYRIDINE ODOR (Pyr). EACH VALUE IS EXPRESSED AS THE RISE (↑) OR NO CHANGE (=) IN TERMS OF THE PROPORTION OF DPM ± 2 3 SE ABOVE OR BELOW THE BASELINE VALUES OBTAINED IN THE CONTROL PERFUSIONS

Animal	Hypothalamic Site	Experimental Condition Feeding	Odor
244a	Ventromedial N	↑	↑(PB)
11a	N Accumbens	↑	↑(PB)
12	Lateral ventromedial N	↑	↑(PB)
9	Medial preoptic area	↑	↑(PB)↑(Pyr)
244b	Basal ventromedial N	=	=(Pyr)
11b	Lateral preoptic area	↑	↑(PB)↑(Pyr)
264	Dorsomedial N	↑	↑(Pyr)
251	Paraventricular N	↑	↑(Pyr)
256	Lateral hypothalamic area	=	=(Pyr)

rat—peanut butter freshly spread on a wooden spatula or a cotton-wool surgical sponge soaked with pyridine. In each control experiment, neither the odorant was presented nor was food offered as the rat's hypothalamus was perfused. Ordinarily, the test stimulus was presented in a random order during experiments which were spaced at 24 hour intervals. In seven animals, feeding and one of two odor conditions were tested. In two rats, however, the ^{14}C efflux was sufficiently stable to enable the second odor condition to be presented (Table 1).

To estimate the relative content of the NE metabolites, hypothalamic perfusates were selected which were obtained during the control and each of the three experimental conditions: feeding, pyridine and peanut butter odors. Each sample was dried under nitrogen and assayed by thin-layer chromatography [8] for NE, normetanephrine (NMN), 3-methoxy, 4-hydroxyphenylglycol (MHPG), 3,4-dihydroxyphenylglycol (DHPG), vanillylmandelic acid (VMA) and dihydroxymandelic acid (DHMA).

RESULTS

To analyze the pattern of NE release, the Hall-Turner method [9] was used in which the DPM value of the sample collected just prior to the 5 min odor stimulus or interval of feeding served as the baseline value from which the change in amine release was calculated. Figure 1 illustrates the proportional efflux of ^{14}C radioactivity under each of the three test conditions: (1) exposure to peanut butter odor (N=6), (2) to pyridine odor (N=4), and (3) presentation of food (N=4). During the control series of perfusion (N=5), the release of NE radioactivity reflected a declining washout curve of radioactivity in the samples collected from individual brain-stem sites. The presentation of either of the two odors evoked a substantial release of the catecholamine which was similar in both time course and pattern of efflux.

Table 1 presents the test conditions under which the efflux of the catecholamine was examined and the hypothalamic sites of push-pull perfusion in nine animals. As

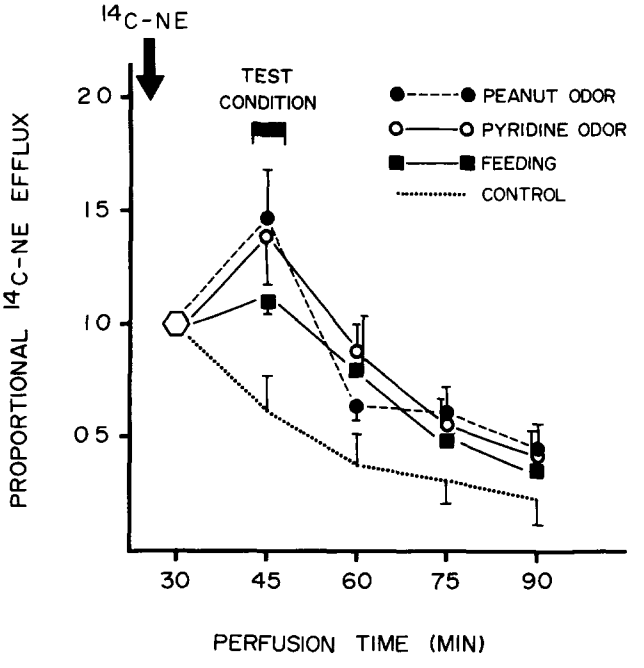

FIG 1 Mean proportional efflux of ^{14}C-NE (± SE) radioactivity from perfusion sites that are denoted as *Immediate Release* in Fig. 2. At the 45 min perfusion interval, as represented by the black bar, either the rat consumed food (N=4) or one of two odoriferous substances, peanut butter (N=6) or pyridine (N=4), was placed beneath the cage floor of the rat The DPM contained in the 30 min sample served as the baseline value. The control washout (mean ± 2 31 SE) was obtained identically but without introducing food or the odor stimuli (N=5).

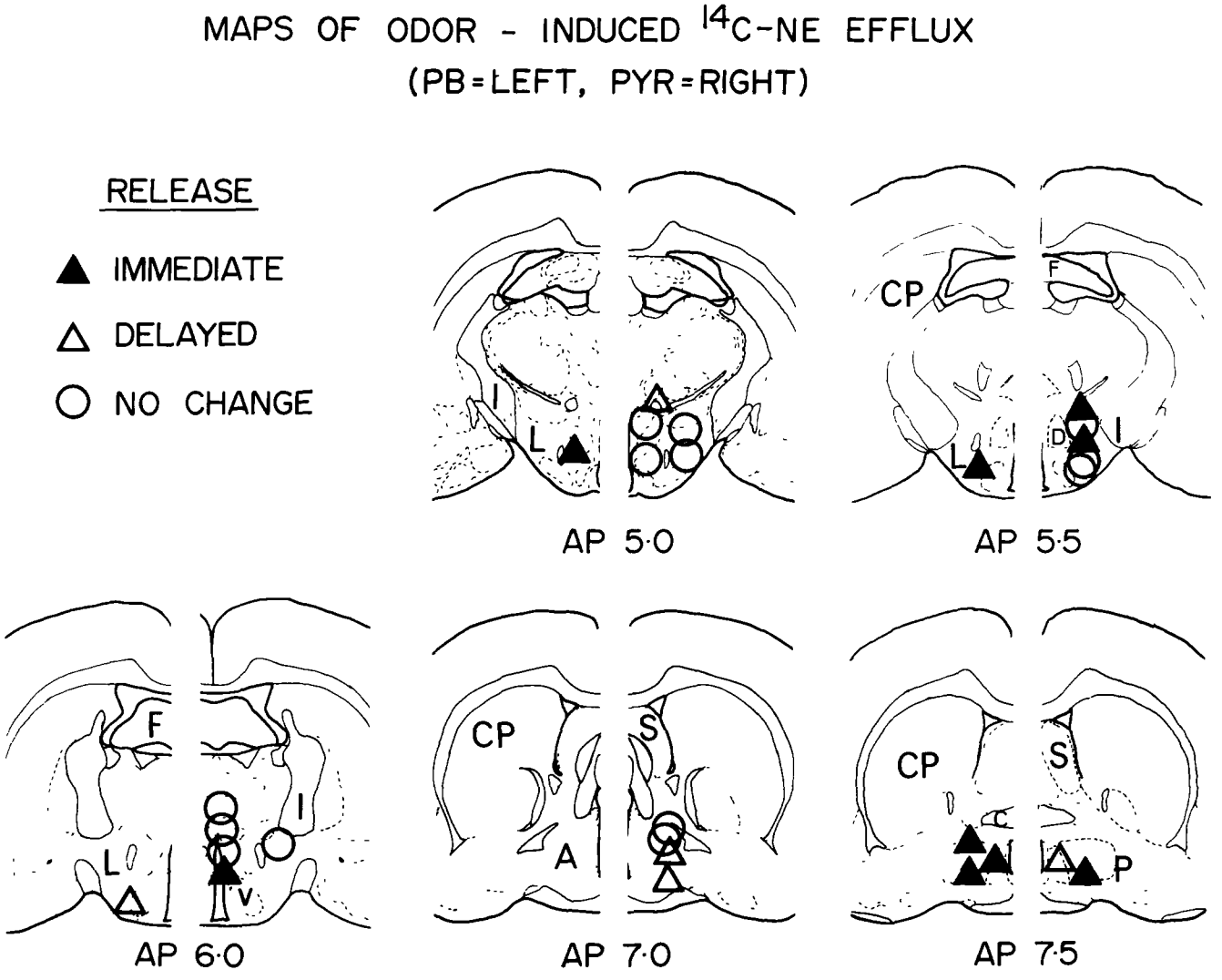

FIG. 2 Anatomical maps in the coronal plane of ^{14}C-NE labeled sites perfused in rats exposed to peanut butter odor (left loci) or pyridine odor (right loci). The solid triangle (▲) denotes an immediate release of ^{14}C-NE during the 5 min test condition; the open triangle (△) indicates a site within which ^{14}C-NE efflux increased but only 15 min or more elapsed after the odor was presented. An open circle (○) denotes a site at which ^{14}C-NE output failed to change when the rat was exposed to the odors.

shown in the Table, the maximal efflux of NE arising as a result of the 5 min test interval of olfactory stimulation was perfectly concordant with the augmented efflux during feeding. That is, within perfusion sites in the preoptic area, i.e., animals 9 and 11b, pyridine, peanut butter odor or feeding evoked a significant rise in NE output ($p<0.01$). Similarly, in each instance in which the ingestion of food failed to evoke NE release, pyridine odor also did not enhance the efflux of the radioactivity.

In order to characterize the anatomical regions within which either the peanut butter or pyridine odor elicited a change in NE activity, a morphological "map" of all perfusion sites was constructed in the coronal plane extending from AP 5.0 through 7.5. Figure 2 presents the results of this mapping in terms of those sites in which a significant efflux (greater than +2.31 standard error) occurred during the test condition (immediate=▲). At other sites NE release was delayed either by 15 or 30 min (delayed=△), a finding consistent with that of Brenells [3] who characterized stimulation-induced output of NE from the olfactory bulb. Efflux of the catecholamine radioactivity less than +2.31 standard errors above the control washout curve is denoted by an unfilled circle (○). As shown in the figure, the sites at which the two odors enhanced the liberation of NE were distributed principally around the preoptic area (i.e., AP 7.0 and AP 7.5) and the ventromedial hypothalamic nucleus (i.e., AP 5.0 and AP 5.5). As illustrated earlier in Table 1, it can be seen that the anatomical distribution of sites of enhanced catecholamine efflux was relatively symmetrical when considered from the bilateral aspect.

Table 2 portrays the changes in the content of NE and five of its major metabolites during both the control and each of the three experimental test conditions. Each value is expressed as the average proportion of the respective ^{14}C activity as

TABLE 2

MEAN PROPORTIONAL CHANGE ± SE IN ^{14}C ACTIVITY OF NE AND FIVE METABOLITES CONTAINED IN PUSH-PULL PERFUSATES COLLECTED DURING THE TEST CONDITION

Test Condition	Metabolite					
	NE	NMN	MHPG	DHPG	VMA	DHMA
Control (N=3)	0.68 ± 0 22	0 76 ± 0.09	0 91 ± 0 14	0 88 ± 0.20	1 36 ± 0.31	1 24 ± 0.21
Feeding (N=3)	1.99 ± 0.15	1 30 ± 0 15	1.37 ± 0.04	1 00 ± 0 85	1.32 ± 0 26	1 26 ± 0 22
Sniffing Peanut Butter (N=3)	3 44 ± 1.85	1.40 ± 0.42	1 94 ± 0 31	1.66 ± 0.77	2.47 ± 1 19	2 07 ± 0 36
Sniffing Pyridine Odor (N=3)	1 47 ± 0.34	1.37 ± 0 12	1.22 ± 0.21	1 01 ± 0 27	2 22 ± 0 23	1.35 ± 0 28

Samples were selected for TLC analysis on the basis of (1) a sufficiently high DPM and (2) a significant change above (+2 31 SE) the control washout curve Perfusates were collected from anatomical sites distributed within the hypothalamus from coronal planes AP 5.0 to 7 5 (Fig. 2). Actual mean DPM 1049 ± 338 (baseline perfusates) and 2158 ± 928 (perfusates obtained during test condition).

determined in the perfusates collected just prior to the test condition as given (Table 2, left column). In each instance, the value of NE was more than double that of the control level. The metabolite values for all conditions were highest, as expected, for VMA and DHMA. The actual DPM value for samples collected before the test conditions was 1049 ± 338, and during the test condition the DPM averaged 2158 ± 928. The mean proportion of radioactivity recovered from the chromatographic plates was 0.47 ± 0.06.

DISCUSSION

Previously we had found in the rat that either the act of eating food or the pressing of a lever to obtain a food pellet causes the release of NE from the animal's hypothalamus [10]. Corresponding to this in vivo result is the fact that a microinjection of NE evokes the motor response to ingest food when the amine is applied to an homologous region of the hypothalamus [1]. These observations have raised the possibility that the activity of this catecholamine in the hypothalamus is related to the stimulation of an efferent pathway underlying food-seeking or consummatory behavior [15,25]. An alternative possibility is that the release of NE is contingent upon the neuronal activation of the motivational system for feeding [10].

The present experiments cast some doubt on both of these alternatives. Clearly, the output of NE from circumscribed sites in the rat's basal forebrain is augmented by an olfactory stimulus. What is particularly significant is the fact that the release of NE and its metabolites is equally as intense in response to the extremely noxious vapor of pyridine as that produced either by the animal's feeding response or by the odor of the highly palatable food. Thus, our results provide further evidence for the idea that a change in hypothalamic efflux of the catecholamine reflects synaptic activity of the afferent pathways to this structure.

Somatosensory stimulation of the rat's forepaw induces an immediate release of dopamine into push-pull cannulae positioned within the ascending nigrostriatal system [19]. In addition, surgical ablation or chemical denervation of the olfactory bulb itself reduces significantly the level of NE or its turnover in the brain-stem as well as olfactory bulb [18,23]. By virtue of a series of morphological mapping, electrical stimulation and enzyme experiments, the medial and lateral hypothalamic cell groups and preoptic area have been implicated in the modulation of olfactory input [4, 20, 22]. In the present experiments, the anatomical sites within which a significant change occurred in amine release were found to correspond in large measure to those regions which contain NE as detected by the dopamine -β- hydroxylase marker [24].

In this connection, it is of interest that the increase in the firing rates of single neurons in the rat's amygdala, olfactory bulb, medial forebrain bundle or hypothalamus cannot be distinguished on the basis of their response to the odor of food or a non-food hydrocarbon [5,21]. Moreover, some single units in the ventromedial portion of the hypothalamus react not only to an olfactory stimulus but also to tactile, gustatory and other sensory input as well [6]. This could help to explain the lack of any differentiation in the nature of the enhanced efflux of NE following the exposure of the rat either to the odor of peanut butter or pyridine.

Thus, the previous observations taken together with our experiments suggest that the term "adrenergic feeding circuit," which has been postulated to traverse the hypothalamus (e.g. [15]), may be a misnomer. In fact, when the rat's intake of food is suppressed by a 6-OHDA lesion of its hypothalamus [17] or is evoked by a microinjection of NE into this same structure, the change in the animal's feeding response could simply be due to a marked shift in the perceived sensory quality of an available food [2,17]. In turn, the degree of paltability would be reduced or enhanced, re-

spectively. Indeed, one main effect of artificially altering the level of catecholamine within the hypothalamus would seem now to be the displacement of the olfactory and perhaps gustatory threshold to a given substance associated with feeding. Further research is required to delineate which sensory modality is primarily affected.

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REFERENCES

1 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.
2 Booth, D. A. and D. Quartermain. Taste sensitivity of eating elicited by chemical stimulation of the rat hypothalamus *Psychon Sci* **3:** 525–526, 1965.
3. Brenells, A. B. Spontaneous and neurally evoked release of labelled noradrenaline from rabbit olfactory bulbs in vivo. *J Physiol* **240:** 279–293, 1974
4. Broadwell, R D. and D M. Jacobowitz. Olfactory relationships of the telencephalon and diencephalon in the rabbit *J. comp Neurol.* **170:** 321–346, 1976.
5 Cain, D P. Effects of insulin injection on responses of olfactory bulb and amygdala single units to odors. *Brain Res.* **99:** 69–83, 1975
6. Campbell, J. F., D. Bindra, H. Krebs and R P. Ferenchak. Responses of single units of the hypothalamic ventromedial nucleus to environmental stimuli. *Physiol Behav* **4:** 183–187, 1969.
7 de Groot, J The rat hypothalamus in stereotaxic coordinates. *J comp. Neurol.* **113:** 389–400, 1959
8. Fleming, R. M and W. G. Clark. Quantitative thin-layer chromatographic estimation of labeled dopamine and norepinephrine, their precursors and metabolites *J Chromatog* **52:** 305–312, 1970.
9. Hall, G. H. and D. M. Turner. Effects of nicotine on the release of ^{3}H-noradrenaline from the hypothalamus. *Biochem. Pharmac.* **21:** 1829–1838, 1972.
10. 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.
11. McCaleb, M. L , R. D. Myers, G Singer and G. Willis. Hypothalamic norepinephrine in the rat during feeding and push-pull perfusion with glucose, 2-DG, or insulin. *Am J Physiol* **236:** 312–321, 1979
12. Meeker, R. B., M L McCaleb and R. D Myers. Changes in catecholaminergic and amino acid activity in the hypothalamus during feeding *Proc Sixth Int Conf Physiol Food and Water Intake,* Jouy en Josas, France, 1977
13 Myers, R. D General laboratory procedures In: *Methods in Psychobiology, Vol 1,* edited by R. D. Myers. London. Academic Press, 1971, pp. 27–65.
14 Myers, R. D. Methods for perfusing different structures of the brain. In: *Methods in Psychobiology, Vol 2,* edited by R. D Myers London: Academic Press, 1972, pp. 169–211.
15. Myers, R. D. *Handbook of Drug and Chemical Stimulation of the Brain.* New York. Van Nostrand Reinhold, 1974.
16 Myers, R. D. Brain mechanisms in the control of feeding a new neurochemical profile theory. *Pharmac Biochem Behav* **3:** 75–83, 1975.
17 Myers, R. D. and G. E. Martin. 6-OHDA lesions of the hypothalamus: Interaction of aphagia, food palatability, set-point for weight regulation and recovery of feeding. *Pharmac Biochem Behav.* **1:** 329–345, 1973.
18. Nakamura, K. and K. Nakamura. Changes in brainstem monoamine turnover after surgical and chemical denervations of the olfactory system In: *Chemical Tools in Catecholamine Research,* edited by G. Jonsson, T Malmfors and Ch. Sachs. Amsterdam: North-Holland Publishing Co., 1975, pp. 285–290.
19. Nieoullon, A., A. Cheramy and J. Glowinski. Nigral and striatal dopamine release under sensory stimuli. *Nature* **269:** 340–341, 1977.
20 Price, J. L. and T P. S Powell. An experimental study of the origin and the course of the centrifugal fibres to the olfactory bulb in the rat. *J Anat* **107:** 215–237, 1970.
21. Scott, J. W. A measure of extracellular unit responses to repeated stimulation applied to observations of the time course of olfactory responses. *Brain Res* **132:** 247–258, 1977.
22. Scott, J. W and B. R. Chafin. Origin of olfactory projections to lateral hypothalamus and nuclei gemini of the rat. *Brain Res* **88:** 64–68, 1975.
23. Sobrian, S K. and C. Cornwell-Jones. Neonatal 6-hydroxydopamine alters olfactory development. *Behav Biol* **21:** 329–340, 1977.
24. Swanson, L. W. and B K. Hartman. The central adrenergic system. An immunofluorescence study of the location of cell bodies and their efferent connections in the rat utilizing dopamine-β-hydroxylase as a marker. *J comp Neurol* **163:** 467–506, 1975.
25. Van Der Gugten, J. V and J. L. Slangen. Release of endogenous catecholamines from rat hypothalamus in vivo related to feeding and other behaviors *Pharmac Biochem Behav* **7:** 211–219, 1977