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Dopamine Efflux from the Brain Stem of the Rat During Feeding, Drinking and Lever-Pressing for Food[1]

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MARTIN, G. E. AND R. D. MYERS. *Dopamine efflux from the brain stem of the rat during feeding, drinking and lever-pressing for food.* PHARMAC. BIOCHEM. BEHAV. 4(5) 551–560, 1976. – To determine whether endogenous dopamine (DA) is involved in the control of feeding and drinking, the cerebral activity of ^{14}C-DA was examined in the rat while the animal consumed food or water. A push-pull guide tube was implanted above sites either adjacent to the third ventricle, the anterior hypothalamus or the substantia nigra in each of 41 rats. After the endogenous stores of DA at specific sites were labelled by a microinjection of 0.5 to 2.0 μCi of ^{14}C-DA, an artificial CSF was perfused at half-hour intervals at a rate of 20–23 μl/min in these sites in the food deprived rat. After a ^{14}C-washout curve of radioactivity in the perfusate was derived for successive control samples, the food-deprived rat was offered food or water which was ingested during the course of one or more perfusions. As the rat consumed food, ^{14}C-DA was released in some experiments from circumscribed sites in the nucleus reuniens and the zona incerta. The efflux of ^{14}C-DA from certain sites in the ventromedial and dorsomedial hypothalamus as well as from the substantia nigra also was enhanced as the rat depressed a lever to obtain food pellets. Since ^{14}C-DA was also released from the zona incerta, perifornical hypothalamus, and into the third ventricle as the rat drank water, these results suggest that dopaminergic neurons in the brain stem play some part in the motor component of ingestive behavior rather than feeding per se.

Dopamine Eating Catecholamines Drinking Brain stem amines Hypothalamus Food intake

APHAGIA and adipsia result when dopaminergic fibers in the brain of the rat are destroyed by the administration of 6-hydroxydopamine (6-OHDA), either directly into the ascending nigro-striatal system [27] or into the ventricle after pretreatment with pargyline [31]. Similarly, an electrolytic lesion of the nigro-striatal bundle produces a severe decrement in the rat's consummatory behavior concomitant with the depletion of telencephalic dopamine (DA) [24]. In addition, the turnover of DA increases in the rat that is fasted [7]. On the other hand, the direct application of DA to the perifornical region [9] or an injection of this amine into the cerebral ventricle of the rat [23] elicits only negligible eating behavior.

These experiments with lesions and microinjections support the idea that DA could be involved in a neural circuit modulating feeding or satiety [18]. However, a major issue is whether or not DA functions endogenously in the control of normal ingestive behavior. To examine this question, the DA storage pools in the rat brain were labelled by the intracerebral injection of ^{14}C-DA. Using the technique of repeated push-pull perfusion, the release of ^{14}C-DA was monitored subsequently from DA-rich sites in the brain stem as the rat was either eating ad lib, drinking water, or performing a lever pressing task to obtain food pellets.

METHOD

Each of 41 male albino rats of the Sprague-Dawley strain, weighing between 300–500 g, was housed individually and maintained on a 12 hr light–dark cycle. Unless stated otherwise, water was available ad lib; however, food was available for only 4 hr each day. Twelve rats were trained to depress a lever to obtain a 45 mg Noyes pellet delivered on a FR-6 schedule of reinforcement. Subsequently, each of these animals was placed in an operant chamber for one half of its daily 4 hr feeding period to work for food. Rates of lever pressing were monitored on a

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cumulative recorder and the total number of lever presses was recorded on a digital counter.

Procedure

Surgery. Using methods described previously [15], stainless steel guide tubes cut from 20 ga thin-wall (TW) tubing were implanted in the brain of the rat anesthetized with 35 mg/kg intraperitoneal sodium pentobarbital. The stereotaxic coordinates for the cannula placements based on the DeGroot system [4] were: (1) lateral ventricle, AP = +5.4, L = +1.7, V = +4.0; (2) third ventricle, AP = +5.4, L = 0.0, V = -1.5; (3) anterior hypothalamus, AP = +6.2, L = +0.8, V = −1.5; and (4) substantia nigra, AP = +3.4, L = +2.3, V = -2.3. In 4 animals, one cannula was implanted above the lateral ventricle and another above the site to be perfused. In these animals the ^{14}C-DA was injected into the ventricle contralateral to the perfusion site. This method of labelling, however, did not permit a sufficient amount of the tracer substance to penetrate to the intended site of perfusion. This was especially true at sites distant from the ventricular space. Therefore, a single cannula was implanted directly above the intended site of perfusion in 37 other rats. The cannula fitted with an indwelling stylet was lowered into the brain and fixed chronically in place by packing cranioplast cement in and around the cannula and three stainless steel screws which had been inserted in the cranium. Following surgery, each rat was allowed a minimum of 5 days to recover before it was used in an experiment.

Microinjection procedure. Radioactive dopamine hydrochloride (3,4-dihydroxyphenylethylamine-l-^{14}C, Amersham Searle, specific activity = 56 mCi/mM) and crystalline inulin-^{3}H (Schwartz Mann, specific activity = 140 mCi/mM) were used as the tracer substances. The catecholamine was diluted with an artificial cerebrospinal fluid (CSF) for the rat [16] which was prepared daily. To prevent degradation of the amine, 0.2 mg/ml of ascorbic acid was added to the artificial CSF to lower its pH to 3.6. Expressed as the free base, the concentration of ^{14}C-DA in the stock solution was 3.1 μg/μl/0.5 μCi. A stock solution of ^{3}H-inulin was prepared with a concentration of 70 μg/μl/0.5 μCi. The amount of labelled amine injected to tag the endogenous store of DA ranged from 0.5 to 2 μCi, viz., 3.1 to 12.4 μg of DA.

Before a microinjection, the solution of radioactive substance was carefully backloaded into the injector cannula which was then inserted into the indwelling guide tube. When given by the ventricular route, 0.5 to 2.0 μCi of DA was permitted to flow into the ventricle over a 30–60 sec interval in a volume of 1 to 4 μl. On the other hand, when the nuclide was infused at a site, 0.5 to 2.0 μl of the solution containing 0.25 μCi to 1.0 μCi was injected again in the same interval. For all injections, a 23 ga stainless steel injector needle was connected to a 10 μl Hamilton syringe by a short length of PE-50 tubing and was always lowered 1 mm beyond the tip of the guide cannula. The needle was kept in place for 45 sec to permit diffusion of the solution into the brain [15].

Push-pull perfusion. The concentric push-pull cannulae were modified from a design described by Myers [17] and connected via PE tubing to calibrated 1 ml syringes mounted on an infusion-withdrawal pump (Harvard Apparatus Co., Model 935). The exact rate of the perfusion was monitored constantly by observing the movement of a small air bubble through the pull tube. Immediately prior to each perfusion, the CSF was passed through a 0.22 μM Swinnex Millipore filter and loaded into the pull syringe. At the end of each perfusion the effluent was rapidly expelled into a calibrated centrifuge tube kept in an ice bath.

The first of each 5 min perfusion was performed one-half hour after the injection of the labelled compound. Successive perfusions were then carried out at 30 min intervals. Each perfusate was collected at a rate of 20 to 23 μl/min. If, at any time during a perfusion, an occlusion occurred in the push-pull system or if the perfusate became discolored, the experiment was stopped immediately. A 50 μl aliquot of the chilled effluent was carefully micropipetted into a scintillation vial containing 10 ml of PCS (Amersham Searle) and the radioactivity was counted in a Packard Model 3320 Tri-Carb Liquid Scintillation Spectrometer. The remainder of the perfusate was placed immediately under a stream of nitrogen and dried to the constituent salts. All samples were counted for 10 min and corrected to DPM either by the method of internal or external standardization. If the CPM of the 50 μl aliquot was greater than 500, the lyophilized sample was stored at −10°C for subsequent chemical analysis.

Thin-layer chromatography. To prepare the lyophilized perfusate for thin-layer chromatography, it was resuspended in 25 or 50 μl of 0.01 N hydrochloric acid containing 1 mg/ml of unlabelled DA. If the DPM in a 5.0 μl aliquot of the suspension were greater than 500, a 5.0 μl aliquot was applied to the chromatogram plate but if the DPM were less, 10 μl were spotted. The following standards obtained from Sigma Chemical Co. were dissolved in 0.01 N HCl in a concentration of 1 mg/ml and dried under a stream of nitrogen gas as they were applied to each plate using a 1 μl disposable capillary pipette: (1) norepinephrine (l-arterenol hydrochloride, NE); (2) dl-normetanephrine hydrochloride (NMN); (3) 3-hydroxytyramine (DA); (4) 3-methoxy-tyrosine hydrochloride (3MT); (5) 4 hydroxy, 3-methoxyphenylacetic acid (homovanillic acid, HVA); and (6) 3,4-dihydroxy-phenylacetic acid (DOPAC). To determine R_f values, each standard compound was run by itself on a 20 × 20 cm glass-backed plate coated with 80 micra of Avicel microcrystalline cellulose powder (Merck, Darmstadt) in the two-dimensional process described by Fleming and Clark [6].

After the identification of the standards on the fully developed chromatogram, the spots were scraped off and placed in individual counting vials containing 0.5 ml of water. After the radioactivity was eluted, 10 ml of the counting fluor were added and the samples were counted for 10 min in the spectrometer.

Free feeding. The ^{14}C-DA washout pattern was calculated from three general areas: (1) the substantia nigra; (2) the third ventricle; and (3) the anterior hypothalamus. Two to three control perfusates were first collected from the food-deprived animal. Five minutes before the next scheduled perfusion, a premeasured amount of dry food was placed in the rat's cage. Subsequently, the animal consumed the food ad lib for the 5 min prior to the perfusion and during the 5 min required to collect the push-pull perfusate. The level of radioactivity in this effluent was compared to that in the control perfusates to determine any release of ^{14}C-DA during feeding.

The food was removed from the cage after the perfusion and reweighed to determine the exact amount of food that had been ingested. Then, additional perfusions were carried

out to complete the ^{14}C washout curve with no food available to the rat. A similar paradigm was followed to examine the effect of drinking on DA release. In this case, water was offered in a calibrated nalgene cylinder to each water-deprived rat instead of food. In several cases the same animal was used for two or more experiments.

Lever pressing. Following either 2 or 3 control perfusions, the rat was placed in the operant chamber and permitted to press the lever for food pellets during a subsequent perfusion. The animal's response rate was recorded on a cumulative recorder as the perfusate was collected. At the end of this perfusion, the rat was either removed from the operant chamber or permitted to continue eating up to and during the collection of the next perfusate. As a control for the specificity of intracellular rather than extracellular release of radioactivity, the washout of radioactive ^{3}H-inulin was also observed during the lever-pressing response after sites had been labelled with ^{3}H-inulin.

Histology. At the end of the experiment, each rat was anesthetized with an overdose of sodium pentobarbital given intraperitoneally. Then 0.9% saline followed by 10% buffered neutral Formalin was perfused retrograde through the descending aorta after the heart had been clamped. The brain was sectioned at 100 micra on a Lipshaw freezing microtome and stained for fibers with hematoxylin according to the method of Wolf [30]. The location of each perfusion site was verified following standard anatomical procedures.

RESULTS

To denote the change in nuclide released during behavior, a proportional measure was adopted since the absolute number of DPM in each perfusate varied from experiment to experiment. The proportional figure was derived by considering the DPM in the perfusate collected immediately prior to the behavioral event as the basal value. Then DPM detected in the perfusate collected before and after that sample were expressed as a proportion of that value. Since the radioactivity in successive samples normally declined, a proportion greater than one derived for the effluent collected during the behavioral event signified an active release of radioactivity above the baseline level.

Anterior Hypothalamus

The release of ^{14}C-DA was not enhanced during feeding in a total of 5 experiments at the 4 rostral hypothalamic sites depicted by the black dots in Fig. 1 (top). Release of ^{14}C-DA refers here not only to the efflux of radioactivity attributable to DA itself but also to the metabolites of this amine. A comparison of the decline in radioactivity following the injection of ^{14}C-DA, during the 5 feeding experiments and the control ^{14}C-DA washout is presented in Fig. 2. The control curve is based on the individual ^{14}C-DA washout curves derived from DPM in effluent collected at the 4 sites shown in Fig. 1 (bottom) from deprived rats which had no access to food. Although the mean intake of food was 2.2 g in the feeding animals, there was no significant difference between the two radioactivity washout curves presented in Fig. 2.

Third Ventricle Sites

An increase in the efflux of ^{14}C-DA was observed during

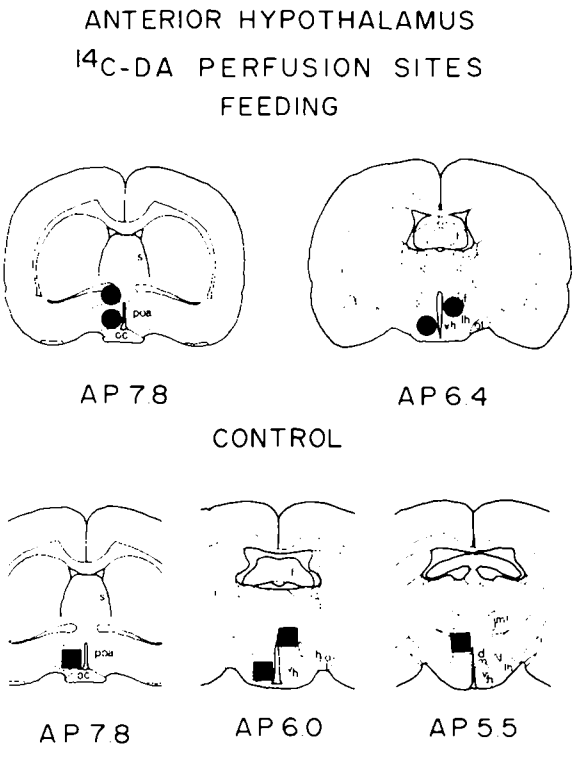

FIG. 1. Histological reconstructions in 5 coronal planes of the sites at which ^{14}C-DA washout curves were derived for both feeding and control animals. The black dot (•) denotes a site at which no increase in ^{14}C-DA release was observed during feeding (top sections). The sites at which non-feeding control curves were derived are denoted (bottom sections) by a square (■). Abbreviations are: dm-dmh, dorsomedial hypothalamus; f, fornix; gp, globus pallidus; lh, lateral hypothalamus, ml, medial lemniscus; mt, mamillothalamic tract; oc, optic chiasm; ot, optic tract; ph, posterior hypothalamus; poa, pre-optic area; pg, periaqueductal gray; re, nucleus reuniens; sn, substantia nigra; v, ventricle; vh-vmh, ventromedial hypothalamus; and zi, zona incerta.

feeding at only one of the five mid-line sites depicted in Fig. 3 adjacent to the third ventricle. At this one site, however, the marked release of ^{14}C-DA was reliably evoked during three different experiments while the animal consumed food pellets. Figure 4 illustrates one of these results for Rat (S-9) which consumed 1.0 g of food as the radioactivity in 50 μl of the effluent increased from 348 DPM in the perfusate collected just prior to the interval of feeding to 829 DPM in the perfusate collected as the animal fed. As shown in the histological reconstruction, the push-pull perfusion site encompassed the nucleus reuniens as well as the paraventricular nucleus.

Figure 5 presents a comparison of the ^{14}C-DA washout curves from those sites shown in Fig. 3 of the nonfeeding control group and the group of rats which were feeding. Overall, the ^{14}C release between the two groups was not significantly different when the proportional data were compared by means of a *t*-test [27].

Substantia Nigra

An increased efflux of ^{14}C-DA occurred during feeding at one of the five sites in the region of the substantia nigra shown in Fig. 6 (top). As shown in Fig. 7, however, feeding

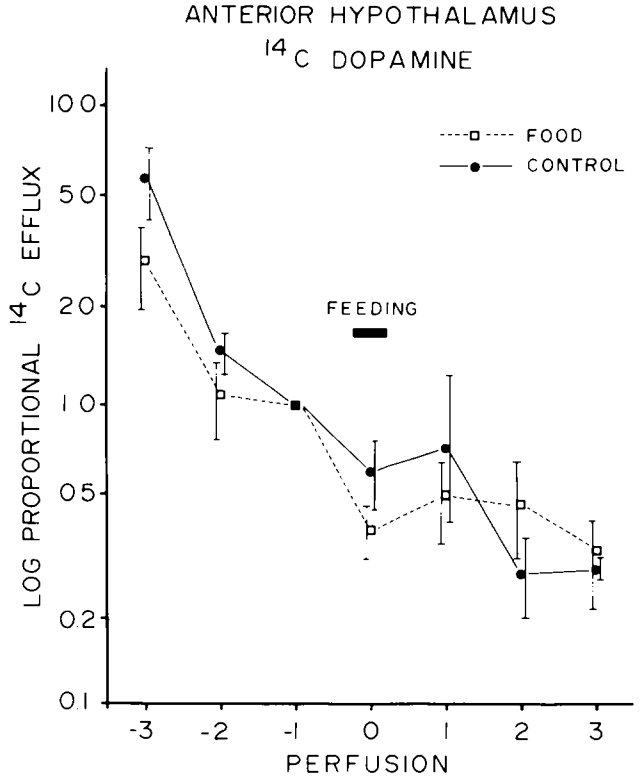

FIG. 2. Mean efflux of ^{14}C-DA (± S.E.) from sites in the anterior hypothalamic area of the rats that were feeding (-□-) and the non-feeding control rats (-●-). The proportional ^{14}C-DA efflux is expressed on a log scale for each perfusion performed at half-hour intervals. The perfusions are numbered relative to the 0 perfusion at which time food was presented (▬). The curves are based on 5 feeding and 4 control experiments.

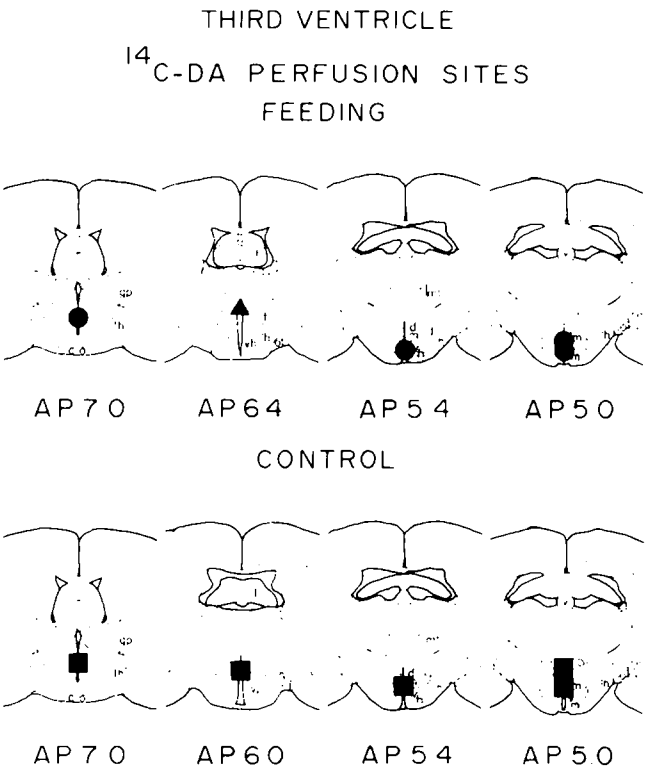

FIG. 3. Histological reconstructions in 8 coronal planes of the sites adjacent to the third ventricle at which ^{14}C-DA washout curves were derived for feeding and control animals. Sites denoted by a triangle (▲) are those from which ^{14}C-DA release was enhanced during feeding, whereas, the dot (•) indicates a site at which no change in release occurred. Control washout curves were derived at the sites denoted by the square (■). Anatomical abbreviations are the same as Fig. 1.

only retarded the washout curve of ^{14}C-DA when the experiment was repeated at this site. A comparison, shown in Fig. 8, of the release curves of the feeding and the control animals for the washout of ^{14}C-DA from the substantia nigra revealed no significant difference between the groups.

Drinking

As the rat drank an average of 3.9 ml of water, the release of ^{14}C-DA radioactivity increased at three of the nine push-pull perfusion sites presented in Fig. 9 in the region of the zona incerta, rostro-lateral and dorsomedial hypothalamus. The typical pattern of ^{14}C-DA release is plotted in Fig. 10 for an experiment during which 3 ml of fluid was consumed as the push-pull perfusate was collected from the site indicated in the inset within the area lateral to the fornix.

Overall Chemical Analysis of Perfusates

In all, 27 of the 526 perfusates collected during this experiment were separated chemically by bi-directional thin-layer chromatography. A typical Chromatogram is presented in Fig. 11. As indicated in Table 1, dopamine represented 37% of the radioactivity recovered in the perfusate 30 min after the cerebral injection of ^{14}C-DA. Subsequently, the level of this amine dropped sharply to 12 and 14% of the effluents collected at 60 and 90 min after the administration of the labelled compound. Conversely, the proportion of homovanillic acid (HVA) increased from 15% of the activity recovered from the chromatogram for the 30 min perfusion to 34 and 40% of the total radioactivity in the two subsequent perfusates. Norepinephrine and other metabolites of DA were detected only in small amounts. The radioactivity contained in the effluent collected beyond the 90 min interval did not permit efficient chromatographic separation of DA from its metabolites using the thin layer technique. Hence, a separation of DA and its metabolites was not possible during behavior. However, the presence of HVA in the previous effluents indicated that the injected ^{14}C-DA was being metabolized via the normal cerebral pathway [10]. The recovery of the ^{14}C radioactivity spotted on the chromatogram was determined to be 34 ± 15% based on 15 determinations of recovery.

Lever Pressing

The release of ^{14}C-DA was detected in 3 of 10 experiments while the rat was depressing a lever to obtain food. As shown in Fig. 12, nine sites were examined for the release of ^{14}C-DA as the rat performed the operant task. The triangles (▲) indicate the sites from which ^{14}C-DA release was observed in at least one experiment, and the

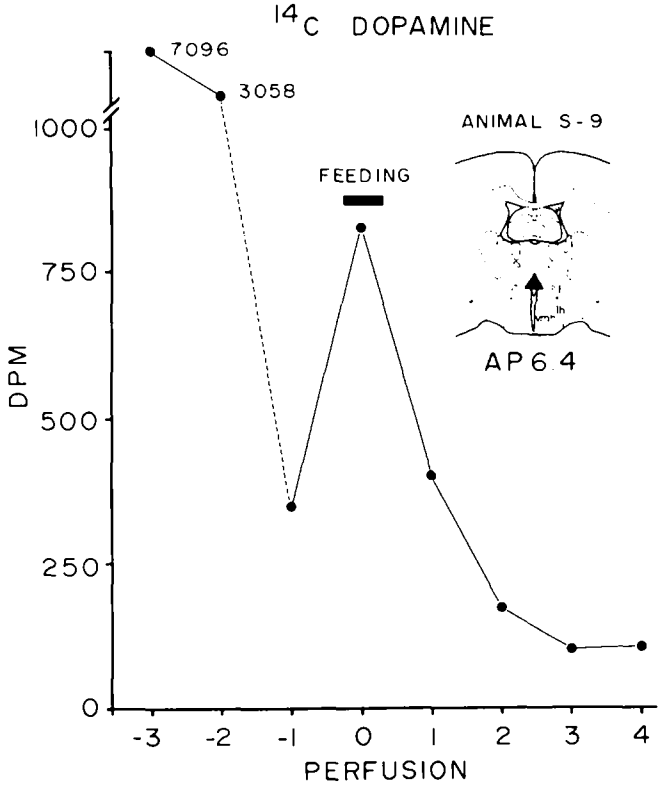

FIG. 4. A ^{14}C-DA washout curve illustrating the DPM detected on successive 50 μl aliquots of the perfusate collected during a 5-min perfusion. The perfusions are numbered as in Fig. 2. Food was presented as indicated by the black bar (▬). The site of perfusion is denoted by the triangle (▲) in the inset.

TABLE 1

THE RELATIVE AMOUNTS, EXPRESSED AS PERCENTS, OF ^{14}C-DA AND ITS METABOLITES (± SEM) THAT WERE DETECTED IN A TOTAL OF 27 PUSH-PULL PERFUSATES COLLECTED AT THE TIME INDICATED FOLLOWING THE CEREBRAL MICROINJECTION OF THE NUCLIDE. THE ABBREVIATIONS ARE THE SAME AS INDICATED IN THE TEXT. NMN WAS LESS THAN 1.0% IN ALL EFFLUENTS

	Latency	(min)	
30		60	90
DA	37 ± 7 (n = 12)	12 ± 2 (n = 4)	14 ± 8 (n = 6)
NE	11 ± 2 (n = 12)	6 ± 2 (n = 4)	2 ± 4 (n = 6)
HVA	15 ± 7 (n = 12)	34 ± 12 (n = 4)	40 ± 9 (n = 6)
DOPAC	3 ± 1 (n = 12)	2 ± 1 (n = 4)	7 ± 4 (n = 6)
3MT	2 ± 1 (n = 12)	1 ± 1 (n = 4)	40 ± 8 (n = 6)
Trailing and Origin	30 ± 9 (n = 12)	46 ± 13 (n = 4)	

black dots (●) denote those at which the release of DA was not enhanced during operant responding.

Mid-Line and Substantia Nigra Sites

As the rat depressed the lever to obtain food pellets,

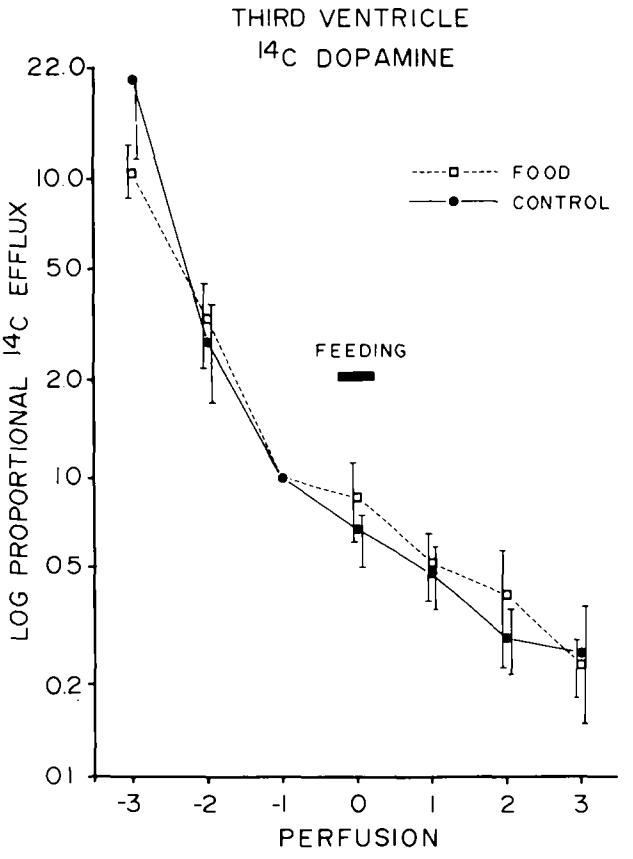

FIG. 5. Mean proportional efflux of ^{14}C-DA (± S.E.) from sites adjacent to the third ventricle. The perfusions are numbered as in Fig. 2 and the black bar (▬) indicates when food was presented. The curves are based on 8 feeding and 6 control experiments.

^{14}C-DA release was evoked from 2 of 6 sites located next to the third ventricle. The washout curves from these two experiments are presented in Fig. 13. On the left, the results of an experiment are shown in which the level of radioactivity in the effluent collected from the dorsomedial hypothalamus increased by 55% as the animal responded on an FR 6 schedule and consumed 19 food pellets during the interval denoted by the black bar (▬). An increase of 63% in the amount of radioactivity recovered in the perfusate was detected in the experiment shown on the right as the rat worked for ten food pellets. The augmented release of radioactivity was not sustained through the subsequent perfusion period even though the animal pressed the lever intermittently for the food pellets during the intervening period.

In one of four experiments, feeding evoked the release of ^{14}C-DA in the region of the substantia nigra. The release was detected in the first perfusate obtained as the animal consumed the food pellets it had received on the FR 6 schedule of reinforcement. Although this rat continued to feed over a 40 min span which encompassed two perfusions, the release occurred only during the first perfusion. All other sites in this region failed to show a significant increase in ^{14}C-DA output during the operant task. The proportional figures for the DPM detected in the push-pull effluents before and during the lever pressing response are given in Table 2 together with the number of pellets consumed during the experiment.

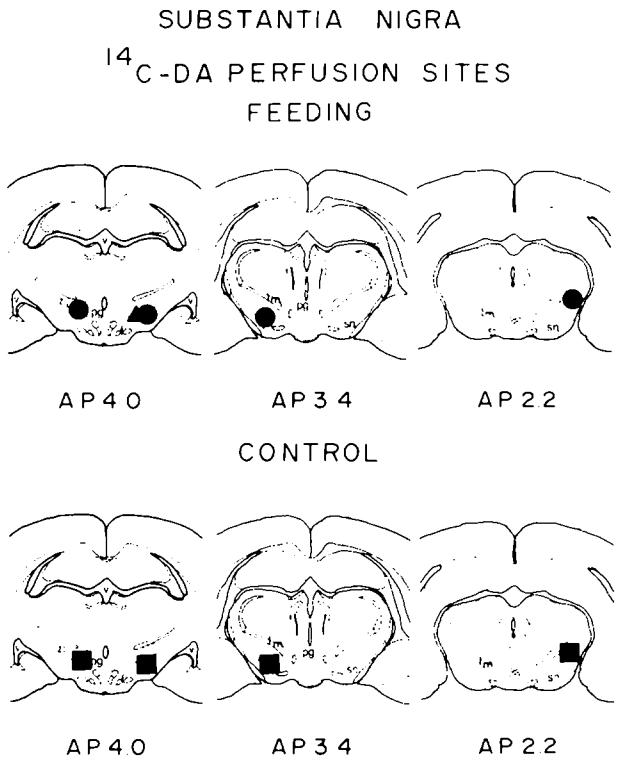

FIG. 6. Histological reconstructions in 6 coronal planes of the sites of perfusion in the region of the substantia nigra from which the washout curves of ^{14}C-DA were derived for feeding and control animals. The triangle (▲) denotes the sites at which the release of ^{14}C-DA was enhanced during feeding and the dot (●) indicates sites from which no increase in the release was detected. Control sites are indicated by the squares (■). Anatomical abbreviations are the same as in Fig. 1.

In six control experiments, ^{3}H-inulin release was not observed during the intervals of lever pressing for food. Table 3 presents the mean amount of release, during the lever pressing, of ^{14}C-DA which was not significantly different from the washout of ^{3}H-inulin ($t = 1.29, df = 14$).

DISCUSSION

These experiments demonstrate that as a rat consumes food, dopamine can be released endogenously in the animal's brain stem but only within certain circumscribed sites. A repeatable release of radioactivity following the intracerebral injection of ^{14}C-DA was observed from one site in the region bounded by the nucleus reuniens in close proximity to the third ventricle. The augmented release of the amine was also evoked by feeding at a site more posterior in the zona incerta. In addition, the release of radioactivity due to ^{14}C-DA was observed at sites in the lateral hypothalamus and at the tip of the third ventricle in the rostral hypothalamus as the rat consumed water. Furthermore, an efflux of radioactivity was observed from the border of the third ventricle near the ventromedial and dorsomedial hypothalamus and also from a site in the substantia nigra, as the animal depressed a lever to obtain and consume food pellets.

Taken together with the previous reports involving 6-OHDA lesions as well as electrolytic lesions [24,27] these results tend to support the concept that dopamine could

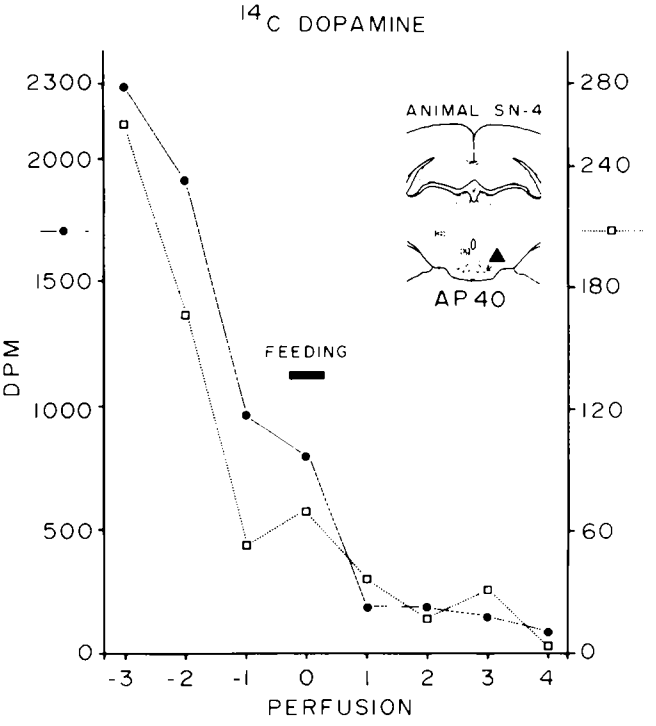

FIG. 7. Two washout curves of ^{14}C-DA activity from the anatomical site depicted in the inset when food was presented (▬) in 2 separate experiments to the same animal. Left and right ordinates correspond to the solid line (-●-) and the dotted line (. .□. .), respectively. The perfusions are numbered as in Fig. 2.

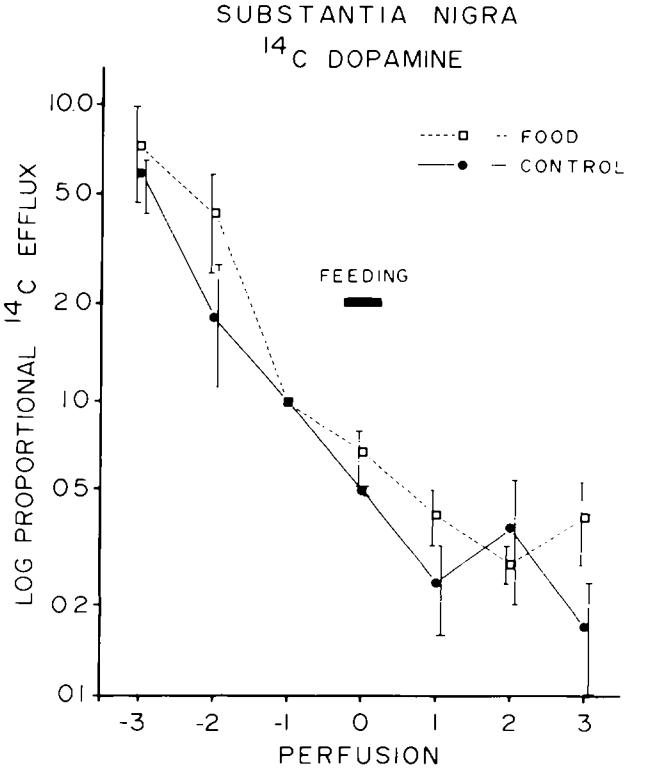

FIG. 8. Mean proportional efflux of ^{14}C-DA (+ S.E.) from sites in the region of the substantia nigra during ad lib feeding. The perfusions are numbered as in Fig. 2. The black bar (▬) denotes when food was presented. The curves are based on 5 feeding and 5 control experiments.

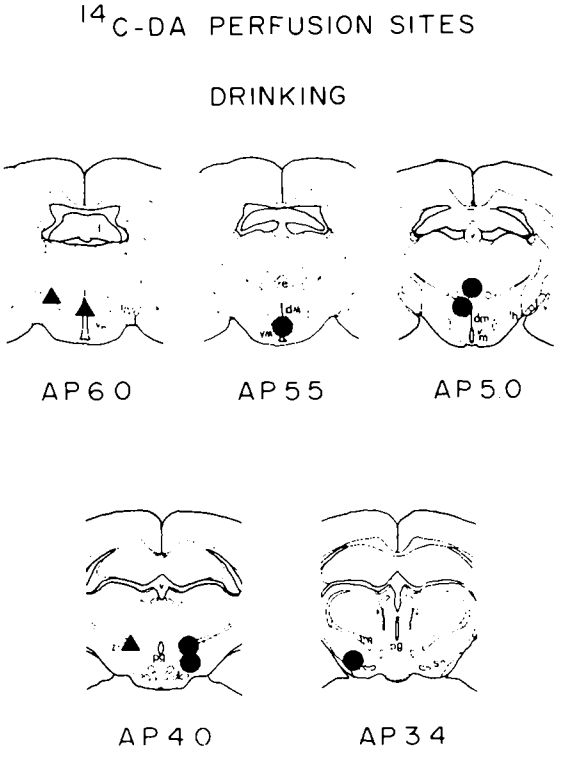

FIG. 9. Histological reconstructions in 5 coronal planes of the sites in the brain at which the efflux of ^{14}C-DA was examined while the rat drank water. An increased efflux was observed at sites denoted by a triangle (▲), whereas no change in release is indicated by a dot (●). Anatomical abbreviations are the same as in Fig. 1.

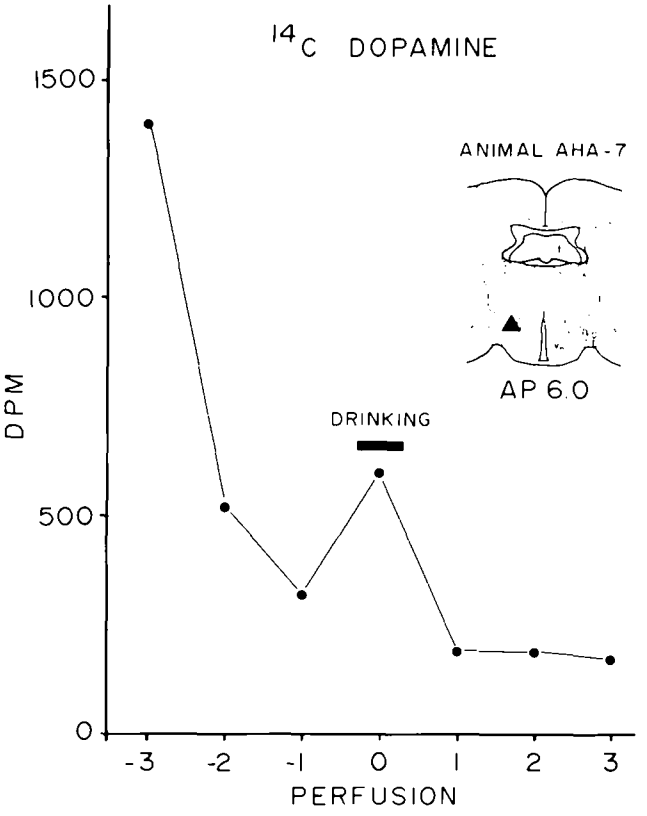

FIG. 10. ^{14}C-DA washout curve of activity obtained from the site (▲) shown in the inset for a water-deprived animal. Drinking of water is denoted by the black bar (▬). Perfusions are numbered as in Fig. 2. DPM were detected in successive 50 μl aliquots of perfusates collected at 30 min intervals.

play some role in the central control of food and water intake. In contrast with the results of a recent series of parallel experiments in which the dynamics of ^{14}C-NE release were examined, the magnitude as well as the frequency of the enhanced output of the catecholamines during feeding is much less for ^{14}C-DA than that which we have observed for ^{14}C-NE [13]. Furthermore, the findings with ^{14}C-DA release are somewhat complicated by the fact that following its intracerebral injection this amine is deaminated readily not only to its major metabolite, HVA, but also hydroxylated to NE. Since Glowinski *et al.* [8] observed that dopamine is transformed rapidly to norepinephrine after its injection into the ventricle of the rat, it is possible that part of the increase in ^{14}C efflux noted during the ingestive response could be due to norepinephrine.

Since the level of radioactivity recovered in the

TABLE 2

THE ^{14}C-DA RADIOACTIVITY DETECTED IN A 50 μl ALIQUOT OF THE PUSH-PULL EFFLUENTS COLLECTED IMMEDIATELY BEFORE AND DURING THE FIRST PERIOD OF LEVER PRESSING. THE PROPORTIONAL FIGURE COMPUTED FROM THESE 2 PERFUSATES. THE NUMBER OF NOYES PELLETS CONSUMED AND THE NUMBER OF LEVER PRESSES EMITTED DURING THE COLLECTION OF THE FEEDING PERFUSATE ARE ALSO LISTED

Animal	DPM Pre	DPM During	Proportion During/Pre	Lever Responses	Pellets Consumed
S-12	86	141	1.63	60	10
BP-1	481	394	0.81	120	20
BP-5	143	222	1.55	114	19
BP-6	139	77	0.55	138	23
BP-2	250	229	0.91	102	17
BP-4	217	67	0.30	126	21
SN-1	519	119	0.23	36	6
SN-5	659	611	0.92	48	8
SN-2	180	341	1.89	186	31
SN-1	1318	360	0.27	48	8

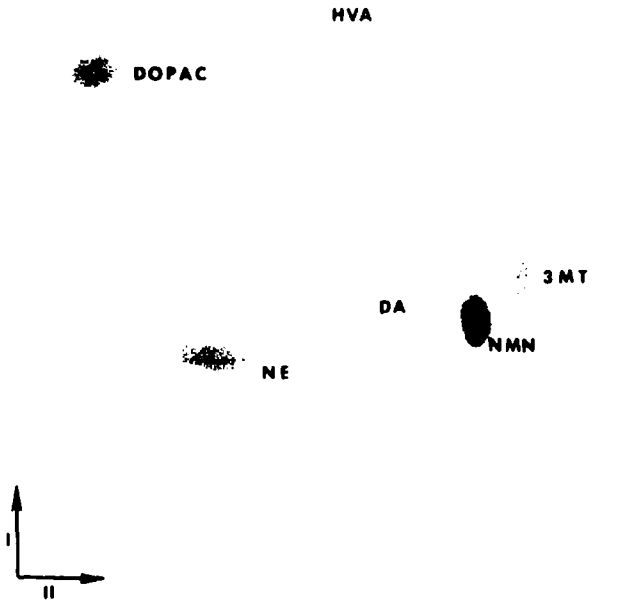

FIG. 11. Thin layer chromatogram with the characteristic pattern of development for dopamine and its metabolites after it had been run in both directions and sprayed with diazotized-p-nitroaniline. Abbreviations are: HVA, homovanillic acid, DOPAC, dopacetic acid; NE, norepinephrine; DA, dopamine, NMN, normetanephrine; 3MT, 3-methyltyrosine.

TABLE 3

THE MEAN EFFLUX OF ^{14}C-DA AND ^{3}H-INULIN DETECTED IN THE PERFUSATE COLLECTED DURING THE LEVER PRESSING RESPONSE. THE EFFLUX IS EXPRESSED IN PROPORTIONAL NOTATION AS DESCRIBED IN THE TEXT (± SEM)

^{14}C-DA	^{3}H-inulin
X = 0.91 ± 0.19 (n = 10)	X = 0.55 ± 0.16 (n = 6)

perfusates was relatively low in relation to our separation techniques, it is difficult to delineate precisely whether ^{14}C-DA or one of its metabolites was being released. Once again, it is possible that the efflux of radioactivity may be due to ^{14}C-NE which we have observed from analagous brain stem sites [13]. Although tagging the brain with radioactive tyrosine and determining the release of radioactivity due to labelled NE or DA during behavior would appear to be a better method for examining which catecholamine is released during behavior, we find that this

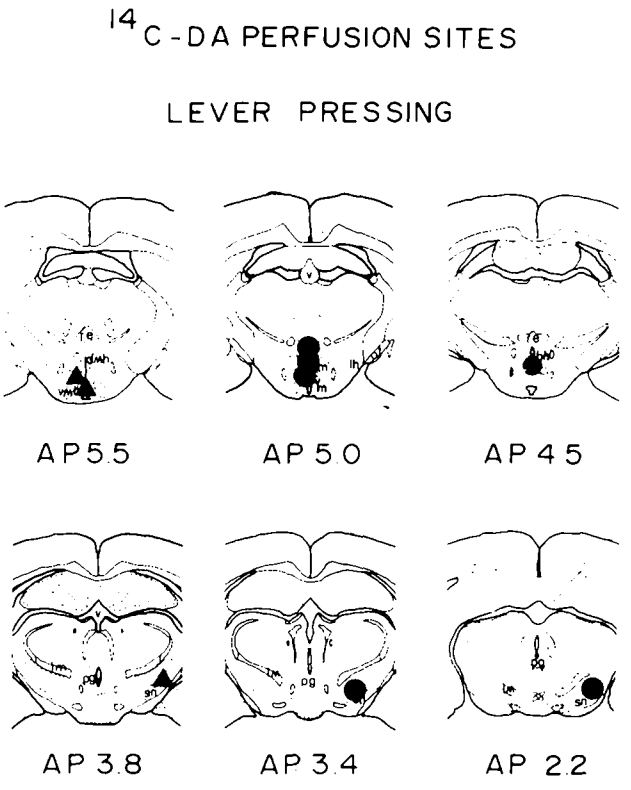

FIG. 12. Histological reconstructions in 6 coronal planes of sites from which the washout of ^{14}C-DA was examined as the rat depressed a lever to obtain food pellets. An increased release of ^{14}C-DA is denoted by a triangle (▲), and no change in release is indicated by a dot (●). Anatomical abbreviations are the same as in Fig. 1.

method does not overcome the separation problem because of the small amount of radioactivity recovered in the perfusate. Future experiments utilizing the new and more sensitive enzymatic techniques [3] in tandem with the push pull procedure [11] may alleviate this problem through direct measurements of the release of endogenous catecholamines.

It is difficult to know which of the behavioral or physiological states underlying ingestive behavior are mirrored by the brain-stem release of ^{14}C-DA. Activation of DA turnover may in part reflect the motor component of either feeding or drinking behavior. The aphagia and adipsia often observed after the cerebral pool of dopamine is depleted by intracranial injections of 6-OHDA [2, 21, 27] would support this view. If such deficits are the result of a partial motor incapacitation, this could explain why pharmacological experiments in which dopamine, injected at norepinephrine-sensitive sites that mediate feeding behavior, has very little overall effect on the animal's feeding or drinking behavior per se [18].

Given by the ventricular route, dopamine elicits only a small amount of eating and after a considerable latency, which could reflect the time taken for the metabolism of the monoamine to norepinephrine [23]. Further, the fact that ^{14}C-DA release was observed during the drinking of water, but the release of ^{14}C-NE was not seen during drinking in previous experiments [13], suggests a functional specificity in the differential release of NE and DA.

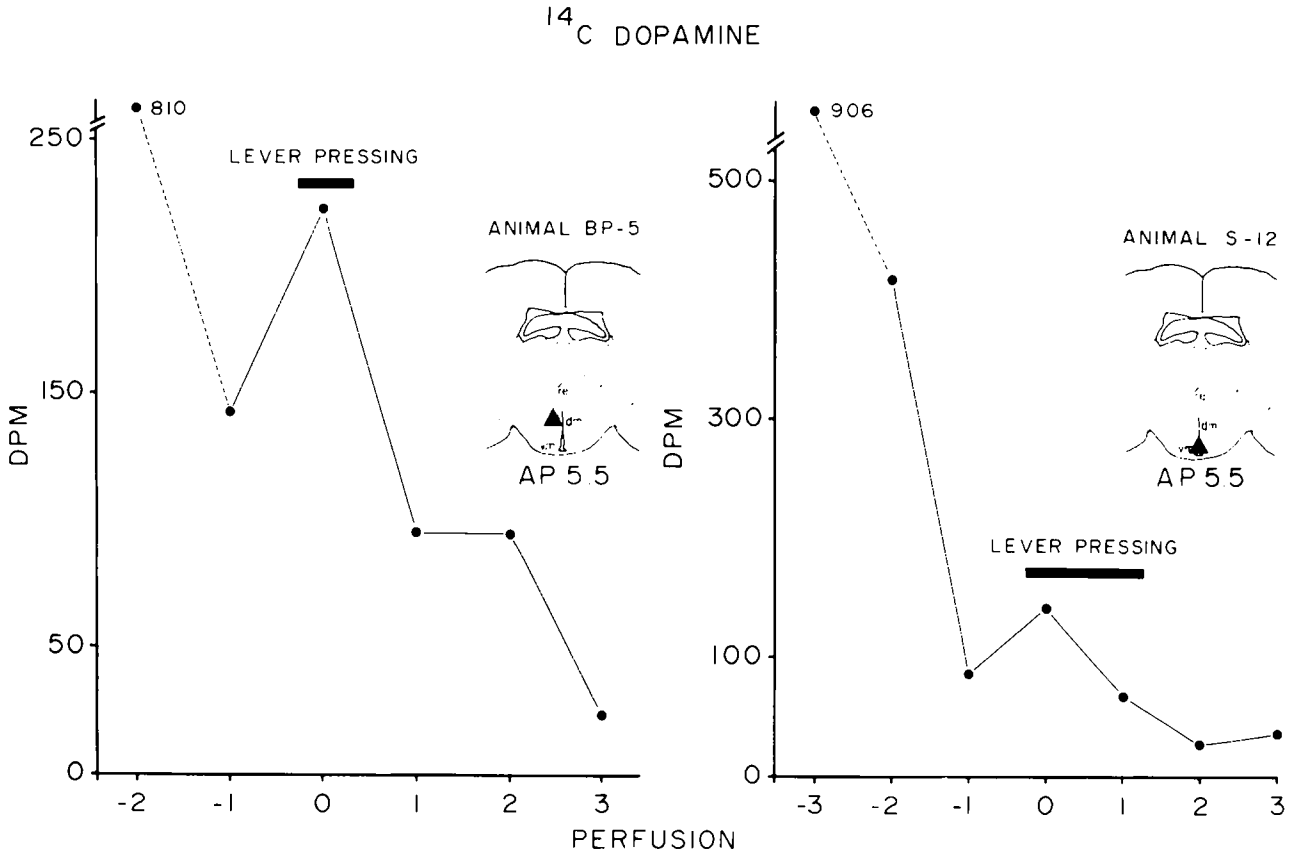

FIG. 13. Two experiments during which an enhanced efflux of ^{14}C-DA occurred at sites of perfusion (▲) shown in the 2 insets as each rat pressed a lever to obtain food pellets. The perfusions are numbered as in Fig. 2 and the interval of lever pressing is denoted by the bar (▬).

Since Ungerstedt reported that severe motor impairment and hypoactivity are part of the sequelae of the central injection of 6-OHDA, DA may be involved in the specific mediation of coordinated movements that are necessary for the ingestion of food pellets. Concordant with this view is the reversal of the motor symptoms by an injection of the DA receptor stimulating drug, apomorphine [27]. Thus, dopamine may play a dual role in the modulation of ingestive behavior. First, it serves as a substrate for the synthesis of norepinephrine, and second, it would seem to exert an effect of its own in the control of the motor responses required for feeding and drinking.

Finally, of special significance to the question of the specificity of release of the labelled amine [18] is the fact that the characteristics of the efflux of ^{14}C-DA were different from that of ^{14}C-inulin during the lever-pressing task. Whereas the activity of the amine can change as a result of the behavior of the rat, the biologically inert tracer, inulin, which is sequestered extracellulary after its injection, simply follows a standard washout curve of activity independent of the animal's response pattern.

At the present time, it is not understood how dopamine and norepinephrine are related to the other chemical factors that have been implicated in feeding and drinking behavior [18] which include: serotonin [5]; amino acids [25]; sex steroids [28]; cyclic AMP [1]; acetylcholine [14,26]; prostaglandins [12] and cations such as sodium and calcium [20,22]. Whether the presynaptic release of dopamine from neurons in the diencephalon is part of the presynaptic mechanism through which these other substances act is not known at the present time. As suggested recently [19], the function of these different endogenous compounds probably lies in the integration of the responses for ingestive behavior which requires not only sensory and motor pathways, but also a system monitoring the nutrients circulating in the blood stream.

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