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Consummatory Behaviors of Hypothalamic Hyperphagic Rats after Central Injection of 6-Hydroxydopamine[1]

DONALD V. COSCINA,[2] CHERYL ROSENBLUM-BLINICK, DAMODAR D. GODSE AND HARVEY C. STANCER

Section of Neurochemistry, Clarke Institute of Psychiatry

and

Department of Psychology, University of Toronto, Toronto, Ontario, Canada M5T 1R8

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COSCINA, D. V., C. ROSENBLUM-BLINICK, D. D. GODSE AND H. C. STANCER. *Consummatory behaviors of hypothalamic hyperphagic rats after central injection of 6-hydroxydopamine.* PHARMAC. BIOCHEM. BEHAV. 1(6) 629–642, 1973.–Two experiments examined the ability of medial hypothalamic lesions to produce overeating, weight gain and finickiness toward palatable and unpalatable solutions in rats with persisting decrements in brain norepinephrine and dopamine due to central injection of 6-hydroxydopamine. In both experiments, such disruption of central catecholamine systems did not significantly reduce overeating and weight gain subsequent to hypothalamic injury. However, acceptance of quinine solutions was increased following 6-hydroxydopamine treatment in both lesioned and non-lesioned rats. The relative importance of brain catecholamines for the expression of different consummatory behaviors is discussed.

Hypothalamic hyperphagia Food intake Finickiness 6-Hydroxydopamine Norepinephrine Dopamine Serotonin

RESEARCH designed to understand better the neural mechanisms which mediate food intake has focused recently on the significance of putative neurotransmitters, particularly the catecholamines (CAs) norepinephrine (NE) and dopamine (DA). This was stimulated in part by Grossman's [22] discovery that direct application of NE to the perifornical region of the lateral hypothalamic area (LHA) produced feeding in sated rats. Shortly thereafter, Heller and Harvey [23] demonstrated that lesions in this general vicinity of the medial forebrain bundle (MFB) produced marked and persisting decrements in whole brain concentrations of NE. Similar brain lesions are well known for their ability to produce aphagia and adipsia in the rat [2]. Independent confirmation of Heller and Harvey's [23] work was reported by Swedish scientists utilizing histochemical fluorescence techniques [18] to visualize endogenous monoamines *in situ*. Anden and co-workers found that the integrity of the MFB was crucial for maintaining intact DA as well as NE systems within brain [3]. More recently, Ungerstedt [45] has reported that selective damage to the nigro-striatal DA system in rats is sufficient to cause the aphagia and adipsia characteristic of LHA-MFB damage. While it is not yet possible to state precisely which brain amine system(s) is responsible for various aspects of consummatory behaviors, it seems clear that these two CAs play some important role in the expression of feeding.

It is well documented that damage to the medial hypothalamus (MH) in the vicinity of the ventromedial nuclei (VMH) produces hyperphagia, obesity, and finickiness toward sapid and noxious food-stuffs (see [25] for review). Since the integrity of brain CA systems seems important for the expression of normal feeding, we wondered if disrup-

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[2] Requests for reprints should be sent to Donald V. Coscina, Section of Neurochemistry, Clarke Institute of Psychiatry, 250 College St., Toronto, Ontario, CANADA M5T 1R8.

tion of both NE and DA neurons would alter the ability of MH lesions to produce hyperphagia and/or finickiness. This question seemed especially interesting since it has been previously suggested that MH lesion effects on feeding are mediated via the LHA-MFB [2,35] which, in turn, is dependent upon intact CAs for the normal expression of feeding [40].

If the behavioral consequences of MH lesions on feeding are dependent upon a full compliment of brain CA neurons, then wide-spread destruction of these neurons should attenuate overeating and/or finickiness subsequent to lesioning. To study this possibility, we have employed the cytotoxic agent, 6-hydroxydopamine (6-OHDA). When injected into the cerebrospinal fluid to by-pass the blood-brain barrier, this compound can selectively destroy both NE and DA neurons, resulting in chronic depletion of both monoamines [7, 8, 14, 27, 47]. The data presented in the following two experiments suggest that such drug-induced CA depletions do not prevent overeating and weight gain in rats with MH lesions. On the other hand, such drug treatment does seem to alter responsivity to noxious quinine solutions when water is not available.

EXPERIMENT 1

Method

Animals, apparatus and intake measurements. Twenty female albino rats (Wistar strain; High Oaks Ranch, Ontario) were used. Rats weighed 230 250 g at the beginning of the experiment. All animals were housed separately in cages with wire mesh fronts and bottoms. Cages were located in a colony room with a 12-hr light dark cycle (lights on at 0800 hr) maintained at 23°C (± 1°). Sufficient amounts of fresh Purina lab chow (pellets) were weighed out daily and made available on the floor of animals' cages to sustain ad lib feeding throughout the experiment. Food spillage was determined daily by weighing food fragments which fell on waxed papers placed under cages. Fresh tap water was also available ad lib, except when testing for acceptance of sucrose or quinine solutions. Intake of all solutions was measured daily from inverted 100 ml calibrated water tubes (Wahmann). Body weights of all rats were determined daily to the nearest g.

Surgical and injection procedures. After establishing daily baseline measures of ad lib feeding, drinking and body weights for a week, animals were divided into three groups: rats receiving 6-OHDA (n = 8), rats receiving the drug's vehicle (n = 7), and normal (untreated) rats (n = 5). Rats in the first two groups were anesthetized with sodium pentobarbital (Nembutal, 35 mg/kg, i.p.) after pretreatment with atropine methyl nitrate (2.5 mg, i.p.) and placed in a Kopf stereotaxic instrument. With the mouthpiece set at its lowest extreme (approximately 6 mm below the interaural line), the first group received an intracisternal injection of 300 μg (free base) 6 OHDA-HCl (Calbiochem) in 20 μl of distilled water to which 1% ascorbic acid (wt/vol) was added to retard oxidation. The second group received only the vehicle (20 μl of distilled water with 1% ascorbic acid). All injections were made through a 1/2 in. 26 gauge hypodermic needle attached to a 100 μl Hamilton Microsyringe. Normal rats were not injected. Following these treatments, rats were returned to homecages for daily measures of ad lib feeding, drinking and weight for 50 days.

At the end of the postinjection measurement period, rats which had received either 6-OHDA or its vehicle were again anesthetized with Nembutal and placed in the stereotaxic instrument. Bilateral lesions aimed at the VMH were induced using a Radionics R-F Generator (55°C for 1 min in each hemisphere through a 0.75 mm dia. Radionics Temperature Probe, uninsulated 1 mm from the tip). With the head flat between lambda and bregma, lesion coordinates were: 2 mm posterior to bregma, 0.5 mm lateral to the midline, 0.7 mm above the base of the skull. After lesioning was completed, scalp incisions were closed with wound clips and rats returned to homecages for an additional 50 days of intake and weight measurements.

Acceptance of sapid and noxious solutions. Following the completion of postlesion intake and weight measures, all rats were tested for one-bottle acceptance of 3% sucrose (SUC) and three concentrations of quinine sulfate (QS). Exposure to each test solution lasted 24 hr. Following two days of water (W) measurements, the sequence of acceptance testing was: 3% SUC, W, 3% SUC, W, 0.02% QS, 0.01% QS, 0.005% QS. Following the last exposure to quinine, rats were given fresh water for one week before being sacrificed for lesion assessment and biochemical measurements of brain samples.

Brain dissection and tissue preparation. All rats were sacrificed rapidly by decapitation. Brains were quickly removed from the calvarium and dissected into forebrain and hindbrain portions by a coronal cut from the posterior aspects of the corpora quadrigemina to the posterior aspects of the mammillary bodies. Hindbrains were discarded. Forebrains were examined visually for MH lesions both from the base of the brain as well as following a second coronal section through the medial hypothalamus. Verbal notes of these visual examinations were kept by tape recordings. Following assessment of lesions, forebrain segments were rinsed in cold (5–10°C) isotonic saline, blotted on filter paper, weighed, wrapped in aluminum foil, frozen in liquid nitrogen, and stored at 20°C until fluorometric assays were performed. The time from decapitation until freezing of these brain samples was 2–3 min.

Biochemical assays. Concentrations of endogenous NE and DA were determined 1–3 weeks after sacrifice using the method of Shellenberger and Gordon [39]. Since we [13] and others [e.g., 15] have found that doses of 6-OHDA similar to those used here can affect endogenous levels of brain serotonin (5-hydroxytryptamine or 5-HT), 5 HT assays were performed on other brain samples from nonlesioned rats which had received 300 μg of 6 OHDA as a check for nonspecificity of the drug. It was necessary to perform these 5-HT assays on separate brain tissues since, at the time of Experiment 1, we were unable to simultaneously measure both indole- and catecholamines in the same brain samples. This problem was rectified in Experiment 2 by modifying slightly the extraction techniques. Forebrain concentrations of engodenous 5-HT in the present experiment were fluorometrically determined using the method of Maickel and co-workers [33].

All fluorescence readings were made with an Aminco-Bowman spectrophotofluorometer. Estimates of amine recoveries were obtained from eight to twelve separate determinations of NE, DA or 5 HT either from pure aqueous standards or from homogenates of brains from normal rats to which exogenous pure standards had been added. Consistent results were obtained for all three amines (mean recovery for NE was 92%; for DA was 78%; for 5-HT was 96%; standard errors were 3 8%) so the biochemical

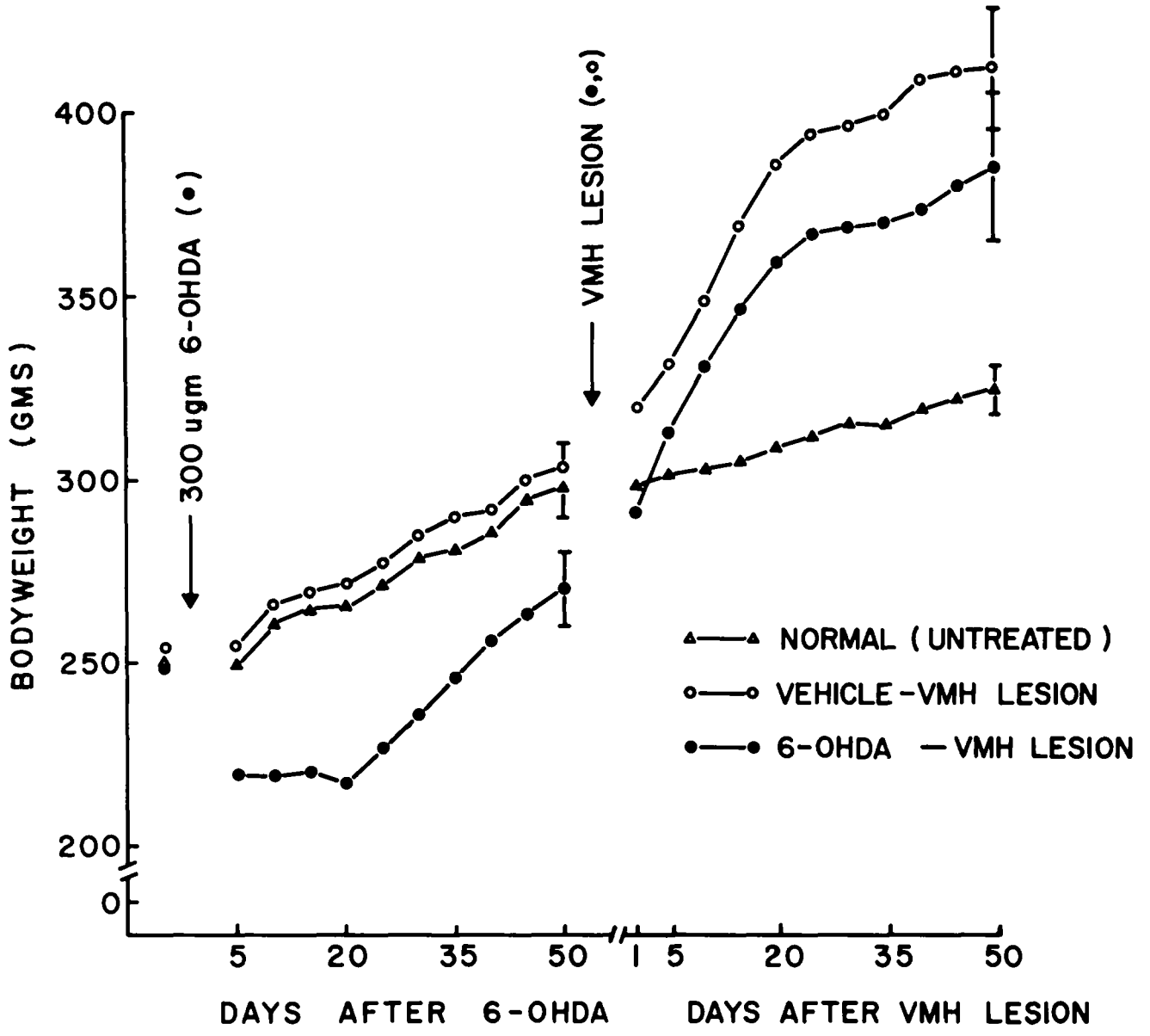

FIG. 1. Body weight in g of rats receiving 6-OHDA and VMH lesions (•), vehicle injections and VMH lesions (○) or no treatments (normals; △). Means are plotted every five days both before and after VMH lesioning. Standard errors of means are provided for Day 50 after 6-OHDA treatment and 50 days after VMH lesioning to allow estimation of group variance.

data obtained from experimental brain samples were not corrected for recoveries.

Statistical analysis. Daily measures of food intake (corrected for spillage), water intake and body weights were computer-analyzed by two-way analyses of variance (ANOVAs) with corrections for unequal ns and repeated observations across days. Separate ANOVAs were performed for postdrug and postlesion data. When indicated, *t*-tests were employed for further analysis of significant F values. Intakes of sapid and noxious solutions as well as amine concentraions were also analyzed by *t*-tests. All tests were two-tailed.

Results

Location of brain lesions. Examination of MH lesions revealed 1.5–2.5 mm destruction in the vicinity of the VMH in five rats treated with 6-OHDA and five rats treated with the vehicle. Lesions in the remaining rats were asymmetrical and/or anterior to the intended sites. On the basis of these observations, postlesion data from these latter animals were deleted.

General appearance of rats after 6-OHDA. After recovering from anesthesia, rats which had been injected with 6-OHDA appeared quiet and somewhat somnolent. Over the next few days, these animals took on the appearance of

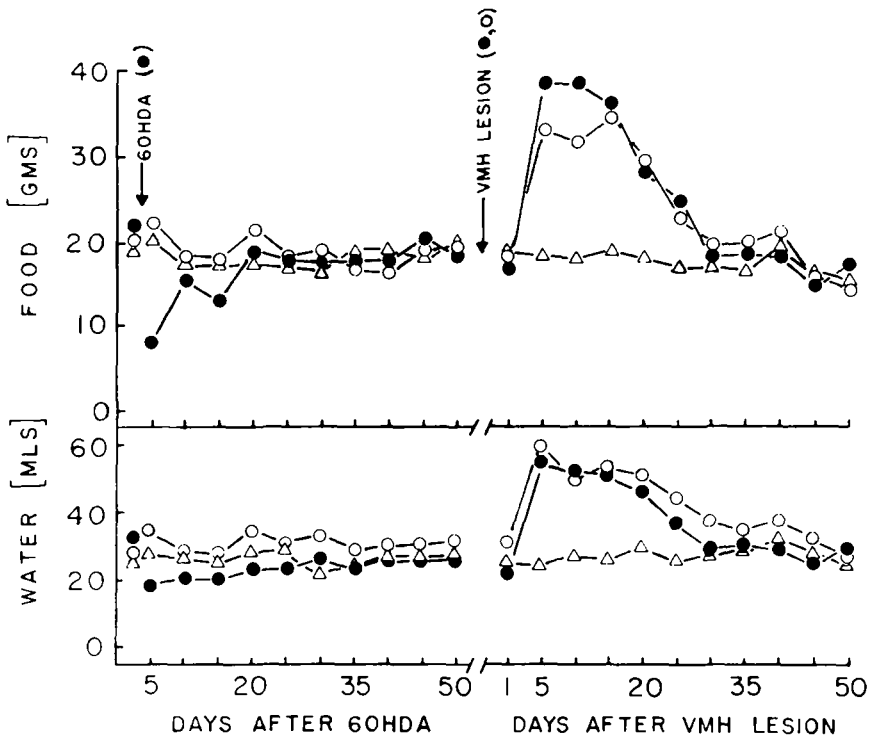

FIG. 2. Food intake (g) and water intake (ml) means for groups described in Fig. 1.

reserpinized or LHA-MFB lesioned rats if left undisturbed in their home cages (i.e., hunched posture, piloerection, lack of grooming, ptosis, catalepsia, sedation; [4,38]). However, when sudden noises were made in the colony room, many of these rats would convulse. Such audiogenic sensitivity has been observed before [6] and may be due to the drug's effects on NE [28]. In addition, drug-treated rats showed rage-like behavior not unlike that of septal-lesioned animals [13,34]. All of these aberrant behaviors began to diminish within 7–10 days after injection. By the end of the second and third postinjection weeks, animals appeared normal. At no time did vehicle-injected controls display any of these behavioral anomalies; indeed, they were indistinguishable from untreated (normal) rats.

Intake and weight measures after 6 OHDA. Concomitant with the behavioral anomalies outlined above, 6-OHDA treatment produced significant decrements in body weight ($p<0.001$) compared to vehicle treatment (see Fig. 1). This weight loss reflected suppressions in feeding and drinking (see Fig. 2) induced by the drug (both $ps<0.001$). However, as with the other behavioral changes mentioned, feeding and drinking increased gradually during the second and third postinjection weeks, resulting in significant weight gain over time ($p<0.01$). By the end of the 50 day postinjection period, food and water intake of 6-OHDA-treated rats was not different from vehicle-treated or normal controls (all $ps>0.05$). However, body weights of 6-OHDA-treated rats remained significantly lower than those of controls ($p<0.01$), reflecting the initial weight loss proximal to drug injection.

Intake and weight measures after MH lesions. After recovery from anesthesia, almost all lesioned rats began eating and drinking regardless of prior treatment. The immediacy of such intake after recovery from anesthesia stands in contrast to the time-course of feeding observed for sham-operated rats, who require significantly longer to begin feeding or drinking [12]. Over the 50 day postlesion period, both 6-OHDA-lesioned and vehicle-lesioned groups ate more food and drank more water than untreated controls (all $ps<0.001$). As can be seen in Fig. 2, these intake differences were apparent during the first 3–4 weeks after lesioning. The maximal amounts of food and water consumed by lesioned rats during this time were roughly twice that of normals' intakes. However, MH lesion effects on both consummatory variables could not be distinguished statistically as a function of drug pretreatment (all $ps>0.05$ between 6-OHDA and vehicle-injected groups).

As a consequence of the lesion-induced hyperphagia and hyperdipsia, both 6-OHDA and vehicle pretreated rats showed significant increments in weight (all $ps<0.001$ compared to normals; see Fig. 1). While the absolute weight gain in grams for drug-treated rats seemed quantitatively less than that for vehicle-treated controls, it must be remembered that the former group weighed significantly less at the time of lesioning. When weight gain was expressed as a percentage increase over body weight at the time of lesioning (see Fig. 3), the ability of MH disruption to produce equivalent weight gain for both groups became more readily apparent.

Acceptance of sapid and noxious solutions. Comparisons of 24-hr (baseline) water intake for two days prior to acceptance testing (see Fig. 4) revealed no difference across

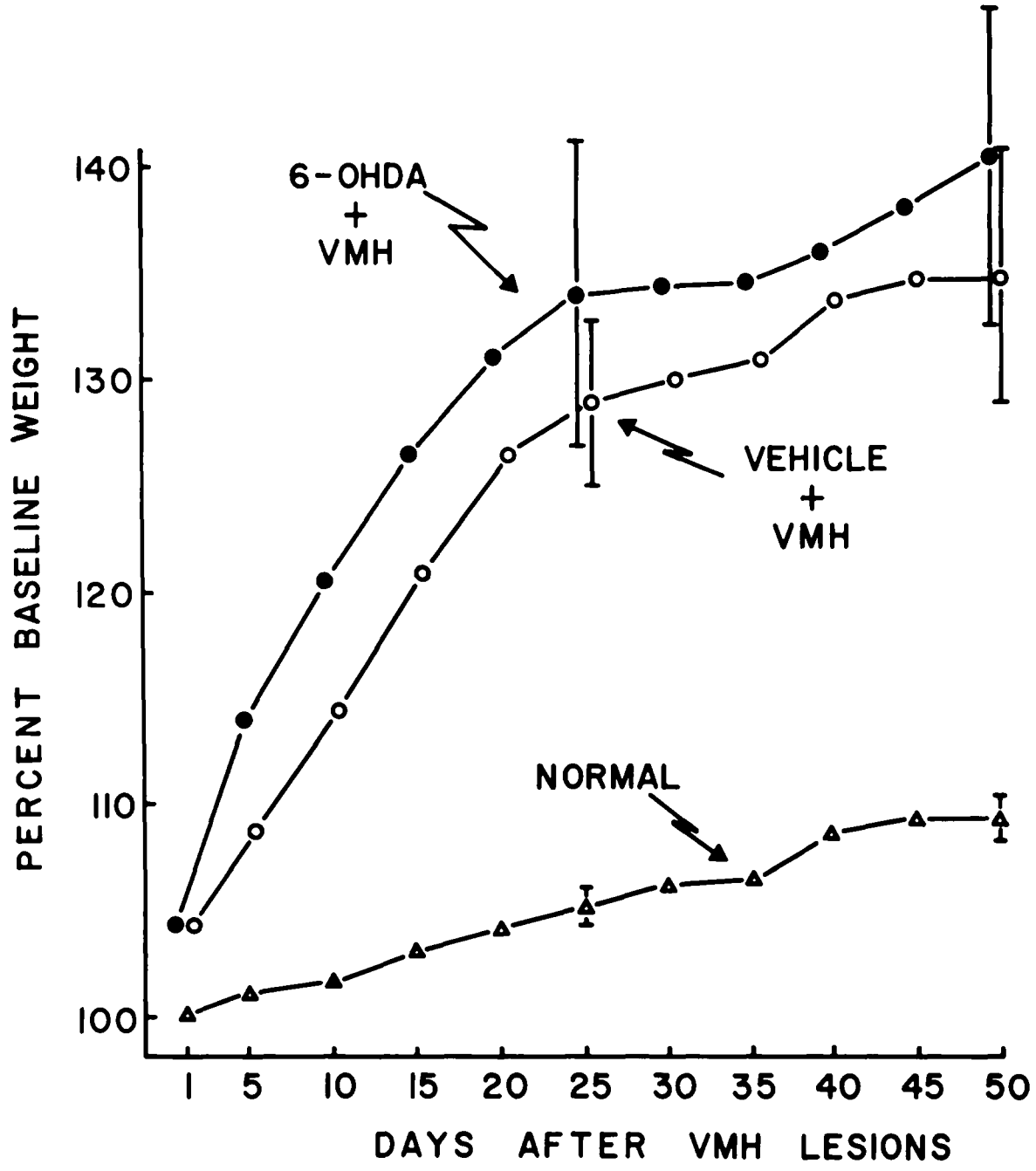

FIG. 3. Body weights of groups described in Fig. 1 plotted as percentages of weight at the time of VMH lesioning, i.e., baseline weights. Standard errors of group means are provided for Days 25 and 50 after VMH lesioning to allow estimation of group variance.

groups ($ps > 0.05$). Therefore, subsequent analyses of fluid consumptions were performed on raw data, i.e., ml intake per solution.

When first exposed to 3% sucrose, all groups showed strong acceptance, drinking about four times more in volume than when water was available (all $ps < 0.01$ compared to baseline). In addition, these drinking increments could not be distinguished across groups ($p > 0.05$). During the second exposure to sucrose, all groups again showed marked acceptance over water ($ps < 0.001$). The intakes of lesioned rats were comparable to those seen during the first sucrose exposure and could not be distinguished as a function of drug pretreatment ($ps > 0.05$ between tests for each group). For some reason, however, normal rats showed even stronger acceptance during this second test. The added drinking increments by normals was statistically higher than

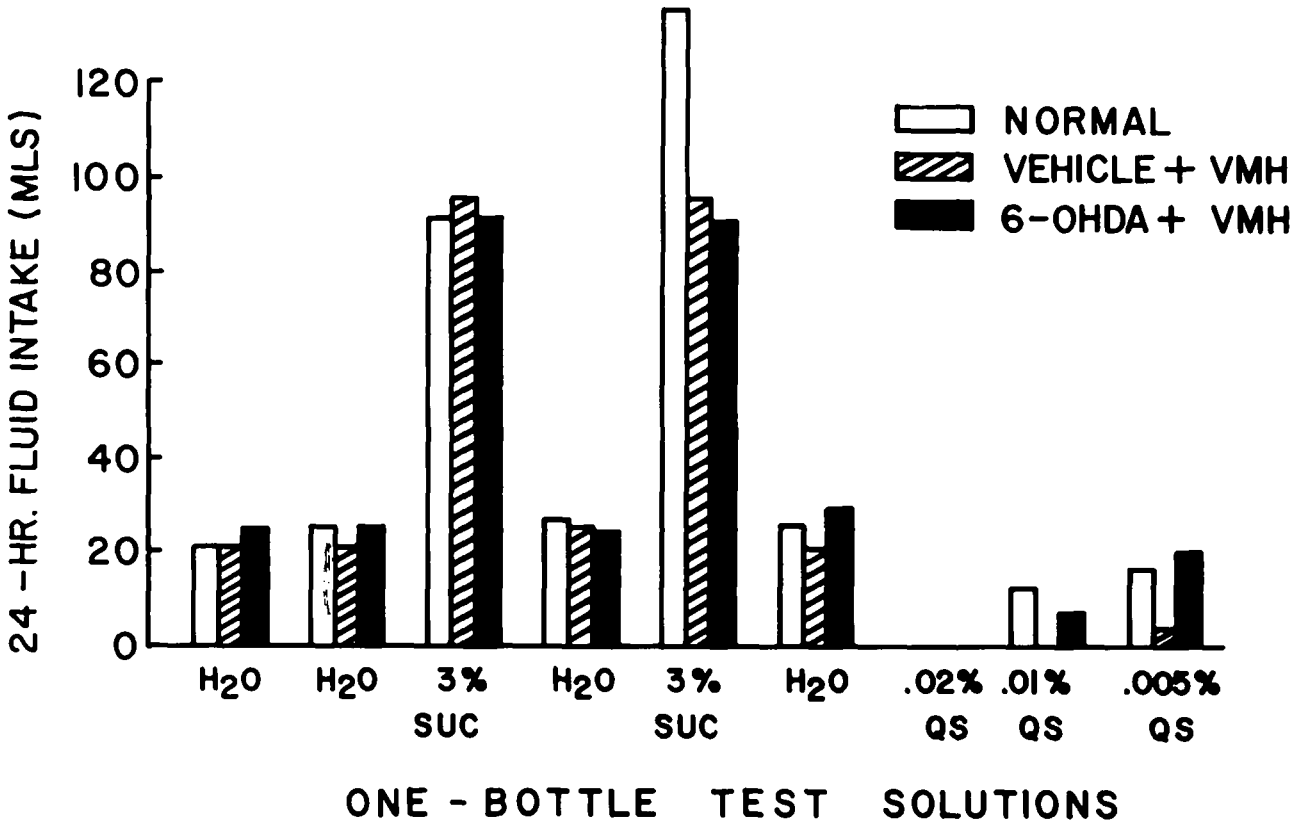

FIG. 4. Mean ml intake (one bottle) of 3% sucrose (SUC) and three concentrations of quinine sulfate (QS). All values represent 24 hr consumptions per group.

that of either lesioned group as well as compared to the intakes of normals during the first sucrose exposure (all $ps<0.05$). The meaning of this increment for normals is unknown.

The pattern of quinine acceptance was quite different across groups (see Fig. 4). At the highest concentration tested (0.02%), virtually all rats refused to drink. On subsequent tests at lower concentrations both normal and 6-OHDA-lesioned rats began accepting quinine (means of 12 and 7 ml, respectively, at 0.01%; means of 16 and 20 ml at 0.005%; $ps>0.05$ between groups for both concentrations). This behavior contrasted with that of MH-lesioned rats pretreated with vehicle, who continued to refuse quinine at the 0.01% concentration. At the 0.005% concentration, only one of five drank, producing reliable ($p<0.05$) group differences when compared to normal or 6-OHDA-lesioned groups for both days.

Concentrations of endogenous amines in brain. Fluorometric determinations of forebrain NE and DA (see Fig. 5) revealed no significant differences between MH-lesioned rats pretreated with vehicle and normal rats (both $ps>0.10$). However, brain samples from MH-lesioned rats pretreated with 6 OHDA contained approximately 60% less NE and 80% less DA (all $ps<0.001$ compared to control groups). Similar injections of 6-OHDA into otherwise normal rats produced only minimal decrements in forebrain 5-HT, i.e., 8-12% less than vehicle or normal controls. Nevertheless, the consistency of these decrements was statistically significant ($ps<0.01$).

DISCUSSION

Central injection of 6-OHDA produced large, chronic decrements in forebrain concentrations of NE and DA while minimally affecting 5-HT. Nevertheless, such marked CA disruption did not substantially alter the magnitude of hyperphagia and weight gain induced by subsequent MH lesions. Such findings imply that the behavioral consequences of MH lesions for ad lib feeding are not clearly dependent upon intact (normal) NE and/or DA systems in brain.

It might be argued that the remaining components of NE and/or DA systems not affected by 6-OHDA were sufficient to maintain normal mediation of MH lesion effects on feeding. This alternative would be more compelling had there been no differences across groups for quinine acceptance. As reported here, only vehicle-treated MH-lesioned rats were finicky toward all concentrations of quinine tested while MH-lesioned rats pretreated with 6-OHDA, along with normal rats, accepted quinine in the two lower concentrations offered. It should be mentioned that the finickiness of vehicle-treated rats with MH lesions cannot be a function of the vehicle per se, but rather reflects the lesion effects. Three additional rats who were not injected with vehicle and became obese following MH lesions (mean bodyweight of 500 g) also showed complete rejection of all quinine solutions when tested at the same time as the other groups. Therefore, the ability of 6-OHDA pretreatment in preventing lesion-induced quinine finickiness seems genuine. The

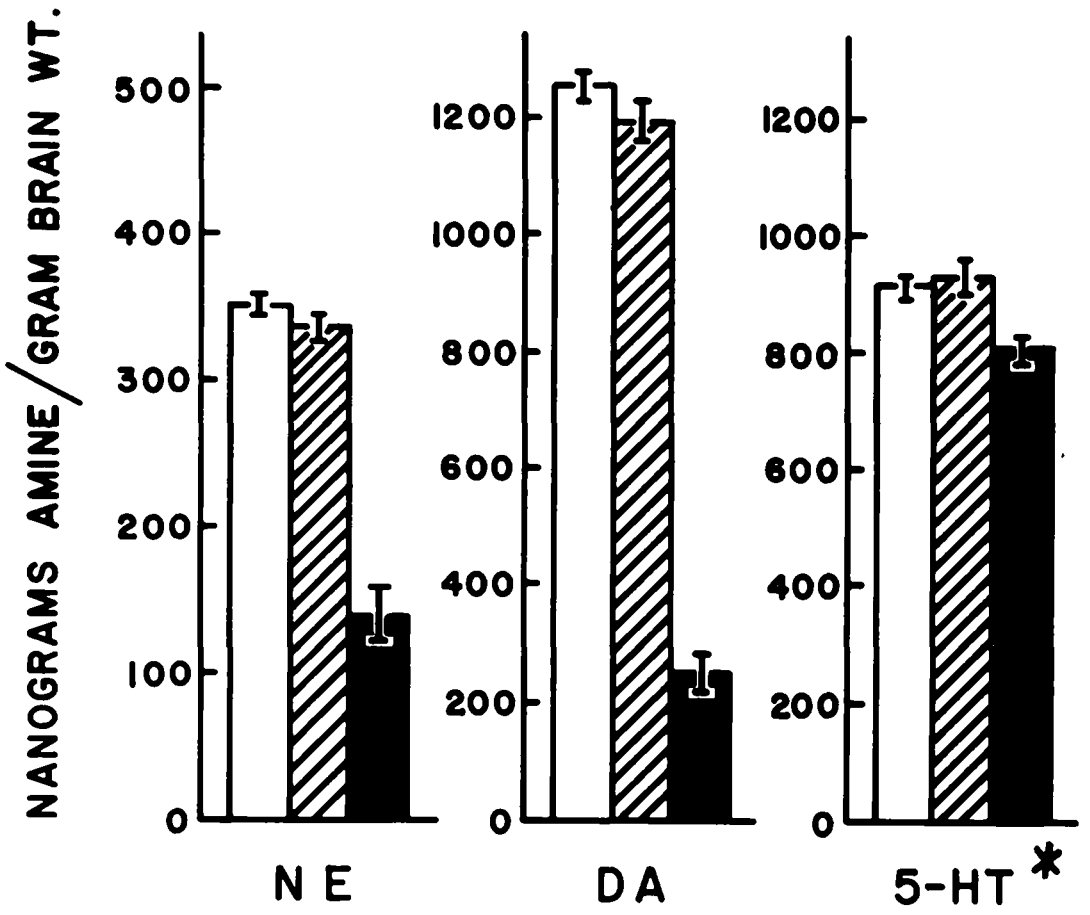

FIG. 5. Means and standard errors of forebrain NE and DA in normal rats (open bar), rats which received vehicle injections plus VMH lesions (hatched bar), and rats which received 6-OHDA plus VMH lesions (solid bar). The asterisk (*) for 5-HT readings indicates that these data were derived from separate brain samples obtained from normal (open bar), vehicle-injected (hatched bar) or 6-OHDA-injected (solid bar) rats, none of which bore VMH lesions.

above data, then, provide evidence that chronic decrements in brain NE and/or DA are sufficient to alter permanently some aspects of consummatory behavior. However, the absence of effect by such CA depletion on hyperphagia and subsequent weight gain following MH injury questions the necessity for the same absolute amounts of these brain amines in mediating overeating.

EXPERIMENT 2

The findings of Experiment 1 suggest that overeating and finickiness may be subserved by functionally different neurochemical systems in brain. The idea that these two behaviors are mediated by neuroanatomically distinct regions is not new. Graff and Stellar [21] made a similar suggestion after observing dissociation of these two behaviors as a function of lesion locus within the MH.

In order to replicate and extend the findings of Experiment 1, we measured food intake and finickiness in additional rats with MH lesions and chronic CA depletion. To allow us to assess the potential significance of recovery of function and/or residual CA functioning in explaining the data in Experiment 1, we reversed the sequence of brain treatments. That is, rats first received MH lesions, were allowed to gain weight, and were later injected centrally with 6-OHDA following deprivation-induced weight loss. Hoebel and Teitelbaum [25] have previously shown that rats with MH injury that become obese will defend their new body weight set point (see [37] for detailed explanation of this concept). In the following experiment, we took advantage of this characteristic to examine the more acute effects of 6-OHDA treatment on overeating induced by food deprivation in MH-lesioned rats.

Method

Animals and apparatus. Thirty-seven rats of the same strain and sex used in Experiment 1 were subjects. Because of difficulties in obtaining rats of the same weight from the commercial distributors at this time, the range of starting body weights was considerably greater (190–360 g; mean of 240 g). All rats were housed and maintained under identical conditions as outlined in Experiment 1.

Surgical and injection procedures, and consummatory variables. Of the 37 rats used, 17 received bilateral lesions

of the MH. Lesion coordinates, surgical procedures and anesthetic agents and their doses were the same as in Experiment 1. However, instead of using R-F heat production to produce lesions, electro-coagulation was used. This procedural change was made in an attempt to induce more restricted MH damage than was possible with the relatively large (0.75 mm dia.) Temperature Probe. The electrode used in Experiment 2 consisted of an insulated nichrome wire (0.17 mm dia.) bared 0.5 mm at the tip. Lesions were made by passing two bursts of 2 m.a. anodal d.c. for 20 sec in each hemisphere. A rectal cathode completed the circuit. Following lesions, rats were returned to homecages for a minimum of 20 days ad lib feeding, drinking and weight measures as described in Experiment 1. The remaining 20 rats (i.e., normals) were not treated but were housed and monitored in all ways identical to lesioned rats.

At the end of the postlesion measurement period, all rats were deprived of food for 7 days. During this time water intakes and body weights were still recorded daily. Following this deprivation interval, all rats were anesthetized with Nembutal and placed in the stereotaxic instrument. Nine of the MH-lesioned rats and 12 of the normals were injected with 250 μg (free base) 6-OHDA-HCl as described in Experiment 1.. The remaining 8 lesioned rats and 8 normals received the drug's vehicle. The lower dose of 6-OHDA used here was chosen in an effort to minimize side effects (see Experiment 1). After central injections, rats were returned to homecages for 45 days of additional ad lib feeding and drinking. Intake and weight variables were recorded for only the first three weeks.

Following the 45 day postinjection period, all rats were tested for finickiness to various concentrations of sucrose and quinine sulfate. As before, each one-bottle test lasted 24 hr. This time, water (W) was made available in between quinine sulfate (QS) tests as well as sucrose (SUC) tests to rule out the possibility that the quinine results in Experiment 1 reflected deprivation-induced thirst motives which might vary as a function of brain treatments. The sequence of acceptance testing was: 1% SUC, W,W, 2% SUC, W, W, 4% SUC, W, W, 8% SUC, W, W, 0.01% QS, W, W, 0.005% QS, W, W, 0.0025% QS. After the last quinine test, all rats were given water for four days, then sacrificed by decapitation.

Preparation of brain tissue, biochemical assays and statistics. Methods for lesion assessments and preparation of brain tissues for biochemical assays were the same as in Experiment 1. In addition to measuring endogenous concentrations of forebrain NE, DA and 5-HT, we also measured 5-HT's major metabolite, 5-hydroxyindoleacetic acid (5-HIAA). This seemed advisable since 6-OHDA treatment may increase 5-HT turnover as indicated by increments in levels of this metabolite compared to endogenous 5-HT levels [30]. Fluorometric assays of 5-HIAA were performed by the method of Maickel and co-workers [33] as were assays for 5-HT. Previous determinations of 5-HIAA from aqueous standards produced consistent and reliable recoveries (mean recovery of 97%, standard error of 6%; six determinations) so the raw data for these indole acid metabolite determinations did not require correction for recovery.

As there was a wide range of body weights for rats in all groups, the postlesion weight changes were statistically analyzed as a percentage of body weight at the time of surgery. Analyses of these data along with food intake (corrected for spillage) and water intake were accomplished by 3-way ANOVAs with corrections for unequal ns and repeated measures across days. Intakes of sapid and noxious solutions as well as amine concentrations were analyzed by separate 2-way ANOVAs with corrections for unequal ns. When necessary *t*-tests were employed for further analyses. As before, all data were analyzed by computer and *p* values reported represent two-tailed distributions.

Results

Location of brain lesions. Of the 17 rats receiving MH lesions, 2 from the 6-OHDA-treated group became ill before completing the entire experiment. Of the remaining 15 lesioned rats, only 10 possessed relatively discrete (1-1.5 mm dia.) bilateral destruction within the MH. Lesions tended to extend more anterior than in Experiment 1. Also, there seemed to be more involvement of the dorsomedial hypothalamic nuclei. Of these 10 acceptable rats, 5 had been injected with 6-OHDA and 5 with the drug's vehicle. Lesions in the remaining 5 rats were assymetrical or unilateral, hence the data from these subjects were deleted.

Intake and weight measures after MH lesions. As in Experiment 1, the majority of MH-lesioned rats began to eat and drink upon recovery from anesthesia. Over the postlesion period, lesioned rats ate and drank significantly more ($ps<0.001$) than normal rats, resulting in significant weight increments over starting body weights compared to normals ($p<0.001$; see Figs. 6 and 7). However, the increments in feeding, drinking and weight were disproportionately greater ($p<0.01$) for lesioned rats which later were to receive vehicle injections. This difference reflected the exclusion of data from the 2 rats who became ill after 6-OHDA administration (see above), thereby lowering the mean intake and weight measures for this group.

Over the 7 days of food deprivation, all rats lost significant amounts of weight ($ps<0.001$ compared to predeprivation weight peaks for each group). However, the percentage of weight lost during this time was comparable across groups ($p>0.10$). By the end of the 7 days of food deprivation, lesioned rats weighed 85% of their predeprivation weight, while controls weighed 83.5% of this weight. In addition, the hyperdipsia displayed by MH-lesioned rats during the ad lib feeding period disappeared over the deprivation interval. Mean intakes dropped to about 15 ml per day. This observation mediates against the possibility that lesioned rats had diabetes insipidus.

Intake and weight measures after 6-OHDA. The use of the slightly lower does of 6-OHDA (i.e., 250 μg vs. 300 μg as used in Experiment 1) had the desired effect. While drug-treated rats were still noticeably irritable for the first few weeks after injection, the incidence of seizure susceptibility as well as the general debilitory effects of the drug reported in Experiment 1 were clearly diminished.

When allowed free access to food after 6-OHDA treatment, MH-lesioned rats (6-OHDA and vehicle treated) ate and drank significantly more ($ps<0.001$) than non-lesioned rats (6-OHDA and vehicle treated). However, 6-OHDA-treated rats (lesioned and non-lesioned) ate and drank less than vehicle controls (lesioned and non-lesioned) ($ps<0.005$ and 0.05, respectively). Perhaps of greater importance, there was a significant interaction ($p<0.025$) between lesion and drug treatments for feeding (see Fig. 7) whereby vehicle-lesioned rats ate more than 6-OHDA-lesioned rats but controls did not vary. On the other hand, no such lesion-drug interaction was found for either drinking (see

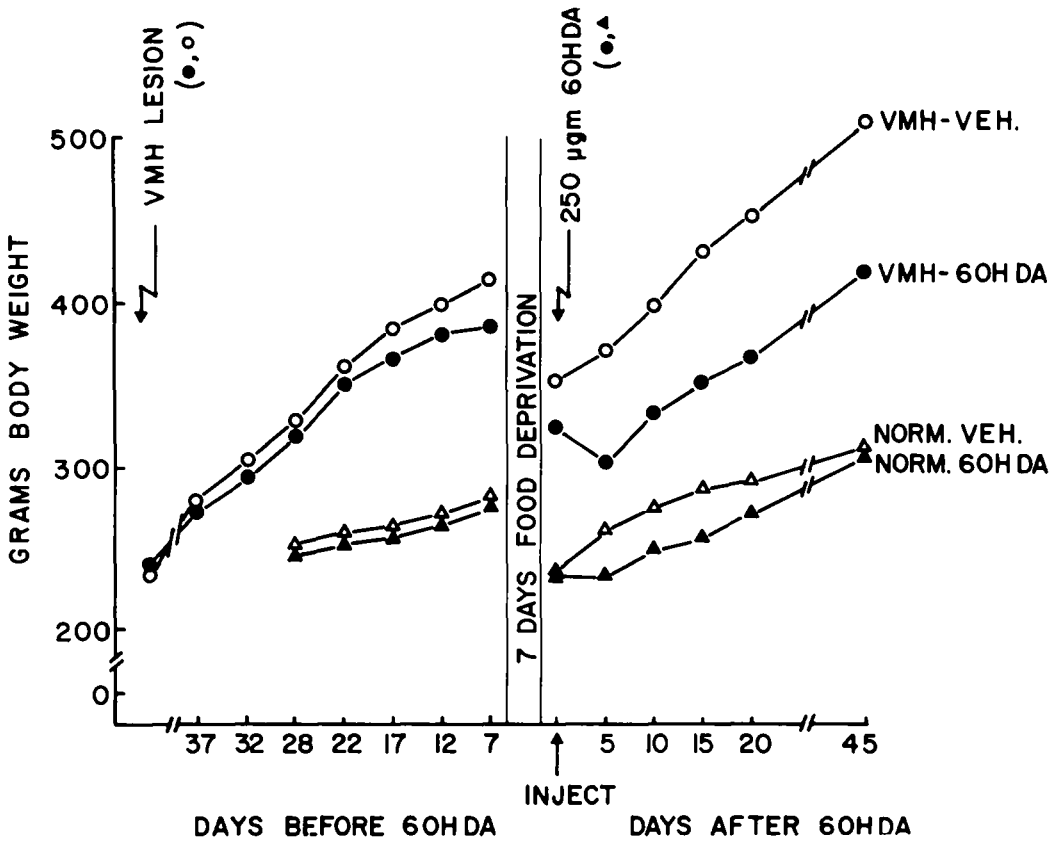

FIG. 6. Body weight in grams of rats receiving VMH lesions and 6-OHDA (●), VMH lesions and vehicle (○), no lesion and 6-OHDA (▲), or no lesion and vehicle (△). Mean data are plotted every five days both before and after 6-OHDA injection.

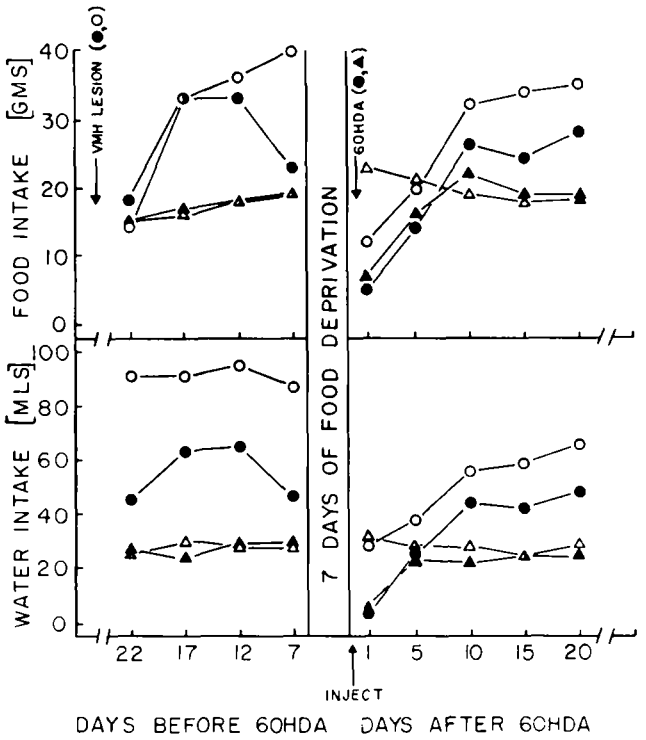

FIG. 7. Food intake (g) and water intake (ml) means for groups described in Fig. 6.

Fig. 7) or weight (see Fig. 6) measures. It seemed incongruous to see only an effect on feeding but not drinking or body weight, especially since drinking must have been food associated (drinking subsided during the 7 days of food deprivation) and body weight should reflect feeding differences. Closer examination of the data resolved this apparent paradox. It will be recalled that prior to food deprivation, lesioned rats which later received the drug's vehicle ate more than lesioned rats which later received 6- OHDA. When this differential intake was corrected for by expressing postinjection feeding data as a percentage of postlesion intake, the feeding differences (i.e., lesion-drug interaction) disappeared. Relative to predeprivation feeding, then, both lesion–6–OHDA and lesion-vehicle groups ate the same amount of food after central injections.

Consistent with the feeding data outlined above, analyses of postinjection body weights as a function of predeprivation weight (see Fig. 8) revealed no significant differences across lesioned and non-lesioned groups for the first three weeks after injection regardless of drug treatment. However, drug treatment per se produced slower weight gain regardless of lesions compared to vehicle treatment per se ($p<0.001$; see also Fig. 7). By Day 45 postinjection, the significance of this drug effect on weight was barely evident ($p<0.05$). Of greatest importance was the lack of significant lesion-drug interactions over the entire postinjection period (all $ps<0.10$).

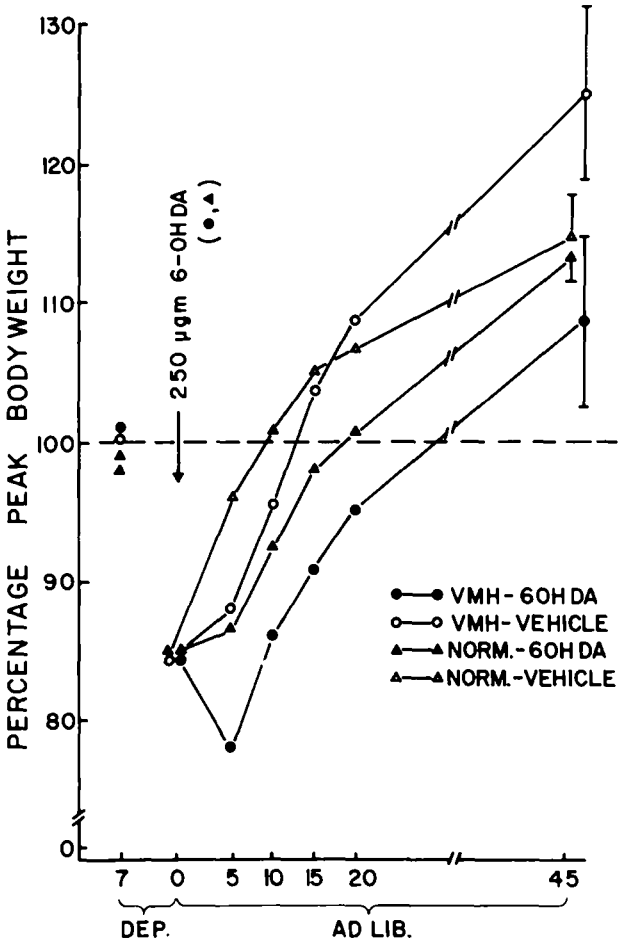

FIG. 8. Body weights of groups described in Fig. 6 plotted as a percentage of weight at the time of 6-OHDA injection, i.e., baseline weights. Standard errors of group means are provided for Day 45.

Acceptance of sapid and noxious solutions. Preliminary analysis of 24-hr water intake just prior to one-bottle acceptance tests revealed that lesioned rats regardless of drug treatment continued to drink significantly more ($p<0.01$) than non-lesioned rats collectively when food was freely available. Therefore, subsequent intakes of sucrose and quinine solutions were analyzed as percentages of this baseline water intake. Due to an accident in the colony room on the day that 4% sucrose intake was tested, data from 80% of all rats were lost. The acceptance data for the remaining three sucrose concentrations are depicted in Fig. 9.

As in Experiment 1, all rats consumed more sucrose than water regardless of concentration tested (all $ps<0.05$ compared to baseline water intakes). However, the degree of acceptance varied as a function of treatments. While there were no intake differences across groups at the 1% concentration, MH-lesioned rats collectively (i.e., 6 OHDA and vehicle treated) drank less 2% and 8% than nonlesioned rats collectively ($ps<0.001$ and 0.005, respectively). In addition, 6-OHDA treated rats collectively (i.e., lesioned and non-lesioned) drank less 8% sucrose than vehicle controls collectively ($p<0.025$). In no case were there significant lesion-drug interactions.

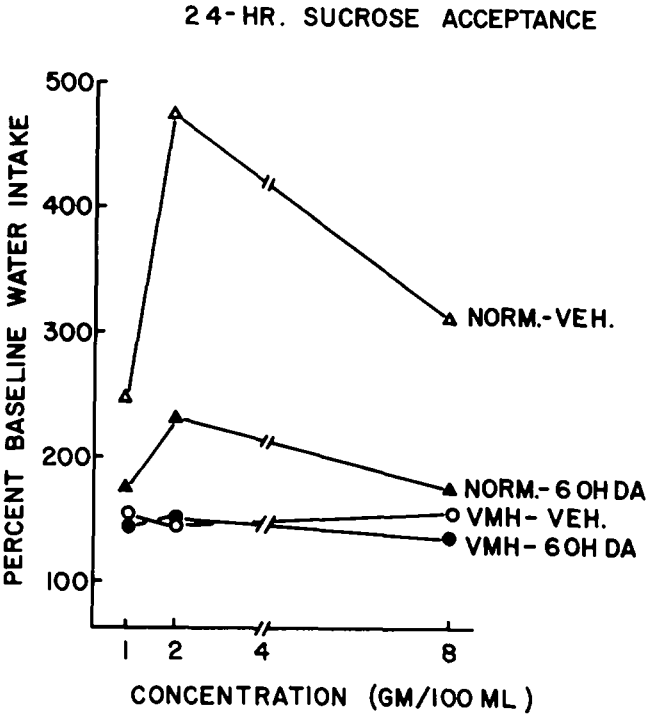

FIG. 9. Mean intakes of three sucrose solutions (1%, 2% and 8%). Each point represents group intakes over 24 hr as plotted from percentages of water intake prior to acceptance testing, i.e., ad lib water intake.

Again, as in Experiment 1, exposure to quinine produced reliable decrements in drinking across concentrations ($ps<0.05$ compared to baseline water intakes). However, these decrements varied as a function of treatments and concentrations (see Fig. 10). At the 0.01% concentration, 6 OHDA treated rats collectively tended to drink more than non-lesioned rats collectively ($0.10>p>0.05$). As the concentration of quinine decreased, these trends became more reliable. At the 0.005% concentration, drug-treated rats drank significantly more ($p<0.05$) than vehicle-treated rats, while lesioned rats continued to drink slightly less ($0.10>p>0.05$) than non-lesioned rats. At the most dilute concentration tested (0.0025%), 6-OHDA treated rats collectively showed clear increments ($p<0.025$) in consumption compared to vehicle controls collectively. At this same concentration, lesioned rats collectively showed statistically significant decrements compared to non-lesioned controls collectively ($p<0.001$). There were no reliable lesion-drug interactions at any of the quinine concentrations tested (all $ps>0.10$).

Concentrations of endogenous amines. Fluorometric determinations of forebrain NE and DA (see Fig. 11) revealed significant decrements ($ps<0.001$) for rats treated with 6 OHDA. Of added interest, these NE and DA decrements were significantly ($p<0.025$) correlated with each other (r between NE and DA was 0.565). Such an observation attests to the similar mode of action by 6-OHDA on CA neuronal systems of both types. In addition, lesioned rats showed small but statistically reliable decrements in NE ($p<0.001$) and 5-HT ($p<0.01$) compared to non-lesioned controls. In no case were there significant lesion-drug interactions. No differences were found across groups for

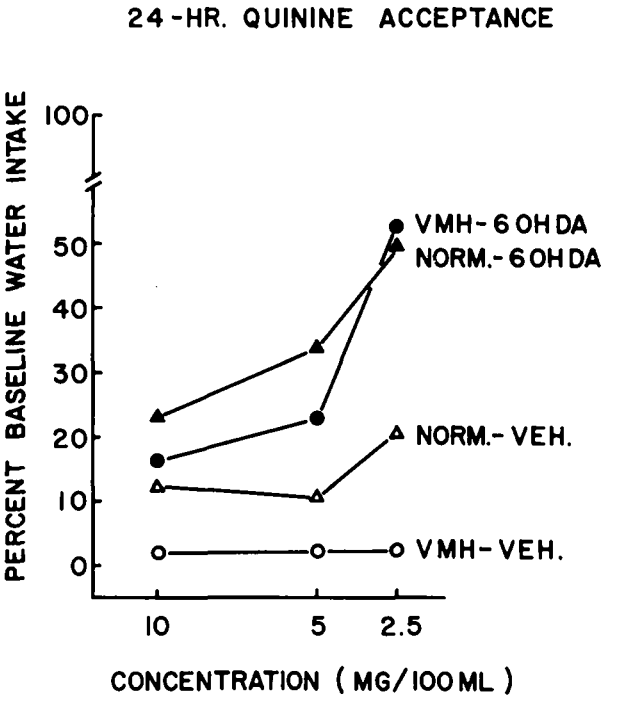

FIG. 10. Mean intakes of three quinine sulfate solutions (0.01%, 0.005% and 0.0025%). Each point represents group intakes over 24 hr as plotted from percentages of water intake prior to acceptance testing, i.e., ad lib water intake.

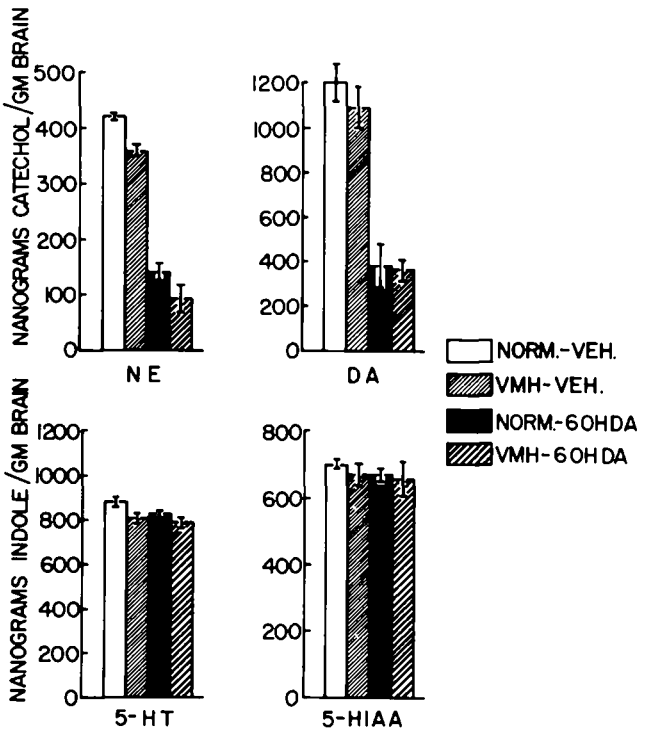

FIG. 11. Means and standard errors of forebrain NE, DA, 5-HT and 5-HIAA (see text for abbreviations). Group designations are indicated in the figure with the following abbreviations: NORM for normal (non-lesioned) rats, VMH for lesioned rats, VEH for vehicle-injected rats, 6-OHDA for 6-OHDA-injected rats.

absolute concentrations of 5–HIAA or for the ratio of 5–HT to 5–HIAA.

DISCUSSION

When allowed to eat freely after 7 days of food deprivation, MH-lesioned rats ate more food and gained more weight than non-lesioned controls. This was so despite chronic depletion of brain NE and DA due to 6–OHDA injection centrally. It must be pointed out that 6–OHDA treatment did retard feeding and weight gain compared to vehicle treatment alone. In addition, a slight but statistically insignificant depression in weight gain was seen for both lesioned groups compared to their respective control groups (see Fig. 8) during the first two weeks post-injection. The observation that 6–OHDA per se decreased feeding, hence weight gain, proximal to injection has been reported before [43,49] and lends support to a general notion of CA involvement in ad lib feeding. Nevertheless, the lack of significant lesion-drug interactions over time in Experiment 2 lends support to the findings of Experiment 1 which implied that overeating and weight gain subsequent to MH injury can occur without a full compliment of brain CA neurons.

Of added interest were the data on sucrose and quinine acceptance. In both Experiments 1 and 2, MH-lesioned rats rejected quinine in concentrations accepted by non-lesioned rats, thus replicating the earlier work of Corbit [10]. On the other hand, chronic depletion of brain NE and DA attenuated the magnitude of this lesion-induced finickiness. A similar increment in quinine acceptance by non-lesioned rats treated with 6-OHDA (Experiment 2) suggests that the integrity of CA neurons per se is important for the expression of finickiness toward such noxious solutions.

The lack of quinine finickiness by lesioned rats with decrements in brain NE and DA does not parallel the ad lib feeding behavior displayed by these same animals. In the latter case, the lesion's effects predominate (i.e., overeating) whereas in the former case, the drug's effects predominate (i.e., less finickiness). This dissociation of lesion-induced consummatory behaviors supports the earlier suggestion by Graff and Stellar [21] of neuroanatomic specificity in the expression of hyperphagia vs. finickiness. What's more, the data here present additional evidence which implicates brain CAs as playing a more crucial role in quinine acceptance than in lesion-induced hyperphagia. In a recent paper, Sorenson, Ellison and Masuoka [41] reported increased finickiness toward quinine solutions following an injection regimen of 6–OHDA which selectively depleted brain NE. Since we observed opposite behavioral effects in the same species with both NE and DA depletion, it may be that the integrity of DA systems in brain represent the crucial difference for the divergence of findings between our data and those of Sorenson *et al.* The possibility also exists that procedural differences (e.g., the use of male rats and testing with quinine HCl by Sorenson *et al.*) are in someway related to these discrepancies. In any event, one fruitful line of approach to this problem would be to determine if differential CA depletion (i.e., NE alone, DA alone, and both NE and DA together) would alter rats' taste thresholds as assessed by two-bottle preference tests. Such work is necessary before any firm conclusions can be drawn

regarding the significance of one-bottle acceptance changes subsequent to 6 OHDA treatment.

The sucrose acceptance data in both Experiments 1 and 2 are difficult to interpret in light of an existing literature which would predict overconsumption of sapid substances after MH damage [e.g., 11, 32, 44]. In both experiments reported here, sucrose acceptance by lesioned rats was similar or deficient to that of controls. These discrepancies between our findings and the existing literature may be due to the use of sucrose in solution rather than in solid food. To our knowledge, there are no reports of MH lesion-induced overresponding to water made sweet by adulteration with saccharin or glucose analogues. On the other hand, the first author here has studied one-bottle intake of both saccharin and glucose solutions before [see 12] and found no differences between MH-lesioned rats and controls. One possible explanation for the lack of significant increments in sucrose solutions after MH injury is that such animals have always been tested in the static phase of hyperphagia, hence are close to their new body weight set point. Therefore, in Experiment 1 here, MH-lesioned rats would not consume significantly more sucrose than normals because it would provide too many calories (see [32]). For rats in Experiment 2, the actual depression of sucrose intakes might simply reflect the fact that test values were expressed as percentages of baseline water intake in order to correct for their hyperdipsia displayed under ad lib feeding and drinking conditions. Such a correction factor could be misleading since hyperdipsic lesioned rats would have to consume more calories present in sucrose solutions in order to maintain percentage intake levels comparable to their elevated baseline water intake.

The significant decrements in sucrose consumption observed for 6 OHDA-treated rats regardless of lesions (Experiment 2) are consistent with a recent report by Breese and co-workers [9]. They found that similar injections of 6–OHDA which depleted both NE and DA in brain produced reliable decrements in 5% sucrose consumption. Additional data from this same paper [9] suggests that decrements in DA alone are responsible for these intake decrements. This suggestion is contrary to the work of Sorenson *et al* [41] who observed decrements in sucrose intake in rats after 6 OHDA treatment which produced only NE depletion in brain. Clearly, additional work is needed to determine the relative importance of these different CAs in sapid fluid consumption. As already suggested above, primary alterations in taste subsequent to 6 OHDA treatment must be discounted before the significance of one-bottle acceptance tests can be adequately assessed. Preliminary work along this line by Breese and co-workers [9] suggests that 6 OHDA treatment effective in depleting both NE and DA in brain does not alter rats' taste acuity to the point where they are unable to distinguish between the taste of water and a solution of sucrose.

The finding in both Experiments 1 and 2 that 6–OHDA treatment per se produces small but statistically significant decrements in forebrain 5-HT concentrations [see also 13 and 15] attests to a slight non-specificity of drug action at the dosage used. However, such depletions were considerably smaller than those found for both NE and DA as would be expected given the primary mode of action for this compound. The finding that MH lesions per se produced small but statistically significant decrements in brain NE and 5-HT (Experiment 2) is puzzling since no such monoamine depletions were found in brain samples from lesioned rats before (Experiment 1). It may be that the more anterior and/or dorsal placements of lesions in Experiment 2 are responsible for these discrepancies. While there appears to be little NE or 5-HT nerve terminals in the VMH region itself, moderate concentrations of both monoamines seem to exist in these other hypothalamic regions [20,46]. More consonant with our findings of NE depletion after MH lesions is a report by Poncy and co-workers [36] who found 50% depletion of whole brain NE after similar hypothalamic injury. However, the increased size of Poncy *et al's* lesions were such that they likely infringed upon ascending noradrenergic neuronal systems within the MFB and/or more anterior or dorsal hypothalamic regions, thus accounting for the magnitude of NE depletion which they observed.

The fact that we found statistically significant decrements in forebrain NE concentrations in MH-lesioned rats might be considered favorable evidence in support of two recent papers [1,31] which imply that NE-depleting lesions of the ascending ventral noradrenergic bundle [see 46] are sufficient to produce hyperphagia. However, since our rats possessed additional depletion of DA as well as NE depletion in areas of brain such as neocortex (unpublished observations), which is innervated by the dorsal noradrenergic bundle (see [46]), it is not possible for us to comment on the relevance of these data to our present report. It is interesting to note, however, that both Zigmond and Stricker [49] and Ungerstedt [45] have suggested that damage to brain DA systems produce profound decrements in feeding. Therefore, it may be that simultaneous damage to NE and DA systems, as reported here following intracisternal 6–OHDA injection, produces animals not unlike rats with combined LHA-MFB and VMH damage [2] in which the LHA-MFB lesion effects (hypophagia due to DA depletion?) predominate at least initially and mask potential VMH lesion effects (hyperphagia due to NE depletion?). If such a neurochemical analogy were true, then combined NE and DA damage would be expected to be less deleterious to processes governing recovery of feeding that would be DA damage alone. While the above mentioned lines of evidence support such a generalization, there is no evidence that overeating can be produced by 6-OHDA injections which selectively lower brain NE alone when the drug is administered through the ventricular system (see [9,41]). Of course, the inability of such treatment to produce hyperphagia may have been due to the lack of specificity given the route of injections and/or the inability to produce substantial NE depletions without the use of multiple ventricular injections.

By way of summary, the results of both experiments reported here question the absolute importance of brain NE and/or DA in the expression of overeating and weight gain subsequent to MH disruption. Our findings, then, seem incompatible with a developing literature [5, 16, 17, 22, 42, 45] which suggests that increments in extraneuronal NE and/or DA seem necessary for the ability to consume food (but see [1 and 31] for alternate suggestions). However, it must be pointed out that while our MH-lesioned rats were depleted of brain CAs by 6–OHDA treatment, such depletion may not have been sufficient to permanently alter certain functional aspects of CA metabolism in remaining CA neurons. It is already known that in spite of persisting decrements in absolute concentrations of NE and DA after

such drug treatment that synthesis and release of these CAs continues to some degree [26,48]. Indeed, it has recently been implied that uptake and turnover in CA neurons may be enhanced following 6-OHDA treatment [29]. It should be pointed out that this same possibility would exist for rats made hyperphagic following NE-depleting lesions of the ascending ventral noradrenergic bundle [1,31]. In keeping with the suggestion of potential increments in CA turnover after 6-OHDA treatment, a recent report by Friedman and co-workers [19] implies that newly synthesized NE and DA are the functional pools important in regulating various aspects of feeding in rat. If the implications of these recent reports are correct, then future experiments designed to clarify the issues raised here and in related studies will be forced to distinguish between old vs. new CAs and/or between bound vs. free amines. In addition, we cannot discount the possibility that changes in post-synaptic receptor sensitivity are related to the behavioral changes observed. Perhaps only when there are adequate methods by which we can assess these additional variables shall we gain insight into the functional aspects of brain monoamine metabolism and the relative roles played by various amine systems in the expression of consummatory behaviors.

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