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Regulatory Deficits after Surgical Transections of Three Components of the MFB: Correlation with Regional Amine Depletions[1]

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MCDERMOTT, L. J., G. F. ALHEID, J. KELLY, A. E. HALARIS, AND S. P. GROSSMAN. *Regulatory deficits after surgical transection of three components of the MFB: Correlation with regional amine depletions.* PHARMAC. BIOCHEM. BEHAV. 6(4) 397–407, 1977. Parasagittal knife cuts along the lateral border of the diencephalon (PS), coronal cuts across the lateral (LMFB) or medial (MMFB) components of the medial forebrain bundle reproduce most of the persisting deficits in responding to glucoprivic and hydrational challenges that characterize rats with lateral hypothalamic lesions or intracranial injections of 6-hydroxydopamine (6OHDA). Each of these cuts produced a different pattern of regulatory deficits, suggesting that individual components of the LH syndrome may be mediated by different neural substrates. This interpretation is supported by the results of our correlational analysis of the relationships between specific behavioral and biochemical effects of our cuts. For example, feeding responses to insulin were reliably correlated with striatal DA concentrations but feeding responses to 2-deoxy-d-glucose (2DG) were not. Water intake during periods of food deprivation was reliably correlated with striatal DA but water intake after an experimental osmotic challenge was not. Only one of the common persisting deficits (impaired feeding response to peripheral injections of insulin) was positively correlated with the duration of aphagia and adipsia.

Medial forebrain bundle, regulatory deficits Medial forebrain bundle, brain amines Dopamine, regulatory deficits Norepinephrine, regulatory deficits Serotonin, regulatory deficits Striatum, regulatory deficits Telencephalon, regulatory deficits Hypothalamus, regulatory deficits 2-Deoxy-d-glucose, norepinephrine Insulin, dopamine Hunger Thirst

LESIONS in the dorsal part of the lateral hypothalamus (LH) produce a complex pattern of changes in food and water intake which is known as the LH syndrome. Following a variable period of aphagia and adipsia, voluntary ingestive behavior reappears. Four stages of recovery and a multitude of persisting deficits have been described [25]. Rats which have recovered from the effects of LH lesions, consume quantities of food and water that are sufficient to maintain a reasonably stable (although lower than normal) body weight. However, the animals are severely hypodipsic or adipsic in the absence of food, eat only palatable diets, and fail to respond to hydrational and glucoprivic challenges which elicit drinking and eating in the intact animal.

It has long been assumed that the LH syndrome results from interference with a hypothalamic feeding and drinking center. This view has recently been questioned by a variety of experimental observations. Lesions in the lateral hypothalamus interrupt a number of fiber systems which connect the basal ganglia and the brainstem. Lesions in the nuclei of origin of these pathways produce severe aphagia and adipsia [13,18]. Knife cuts along the lateral border of the hypothalamus which produced little or no direct damage to cellular components of the area not only produced prolonged aphagia and adipsia [9] but also the full spectrum of persisting deficits that characterizes the recovered lateral rat [10].

Pharmacological investigations have indicated that at least some of the effects of LH lesions may be due to an interruption of catecholaminergic pathways which pass through the area. Intraventricular [22,30,31] as well as intranigral [26], intrahypothalamic [15], and intrapallidal

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[21] injections of 6-hydroxydopamine (6OHDA) (a neurotoxin which preferentially destroys catecholaminergic neurons and their processes) produce aphagia and adipsia as well as many (apparently not all) of the persisting deficits in response to hydrational and glucoprivic challenges which characterize rats with LH lesions.

We [1,17,11] have recently completed a series of investigations of the relationships between the behavioral and biochemical consequences of a variety of knife cuts which interrupt various aspects of the diffuse aminergic projections to striatum and forebrain. The results of these studies indicate that the immediate behavioral effects of these lesions (i.e., the duration of aphagia and adipsia or the magnitude of hyperphagia or hyperdipsia) are related to several complex changes in telencephalic serotonin and dopamine as well as striatal dopamine.

The present series of experiments represent a direct extension of one aspect of this work. We [17] have previously shown that knife cuts which selectively interrupt lateral components of the MFB produce aphagia and adipsia whereas an interruption of the medial components of the MFB result in hyperphagia. The present experiments were designed to examine the effects of these cuts on the response to various glucoprivic and hydrational challenges and to determine whether significant correlations exist between any persisting behavioral impairments and biochemical changes following these knife cuts.

METHOD

Animals

The animals were adult male albino rats of the Sprague-Dawley strain (Holtzman, Madison, WI) which were housed singly in a temperature controlled (21°C ± 2°C) colony. A 12 hr light/dark schedule (lights on at 7 a.m.) was maintained. Except when noted, food (Teklad 6% fat, rat and mouse pellets) and tap water was available ad lib.

Surgery

Surgery was performed under Nembutal anesthesia using a retractable wire knife constructed of 27 gauge stainless steel hypodermic needle stock and a 150 μ diameter spring steel wire which was extended from the slightly bent tip of the guide cannula. One of the 3 bilateral transections was made in the brain of each animal using the coordinates from the de Groot [7] atlas of the rat brain as described earlier [17].

(a) Parasagittal cuts along the lateral edge of the hypothalamus (PS). Fourteen rats received parasagittal cuts along the medial edge of the internal capsule. The knife guide was inserted into the brain so that its tip was placed at AP = 6.0, H = 1.5, L = 2.0. The wire knife was then extended 2.0 mm caudally and lowered to the base of the brain, thus cutting across the laterally coursing fibers of the MFB in a plane extending from the thalamus to the base of the brain and from the anterior hypothalamus to the mammillary bodies. Ten animals were used for behavioral testing and biochemical assays and four were used for histological confirmation of the placement of the cut.

(b) Coronal cuts across the fibers of the lateral MFB (LMFB). Thirteen rats received coronal cuts across the lateral fibers of the MFB. The knife guide was inserted into the brain so that the tip was placed at AP = 4.6, H = 1.8, L = 3.0. The wire knife was then extended 1.2 mm toward the midline and lowered to the base of the brain, thus cutting across lateral components of the MFB as they course through the internal capsule just anterior to the mammillary bodies. Seven rats were used for behavioral testing and biochemical assays and six were used for histological confirmation of the placement of the cut.

(c) Coronal cuts across the medial fibers of the MFB (MMFB). Twenty-one rats received coronal cuts of the medial fibers of the MFB as they course through the lateral hypothalamic area just anterior to the mammillary bodies. The knife guide was implanted into the brain so that its tip was placed at AP = 4.6, H = -2.0, L = 2.0. The wire knife was then extended 1.2 mm toward the midline and lowered to the base of the brain, thus transecting fibers in an area bounded by the internal capsule, fornix, zona incerta and base of the brain. Seventeen rats were used for behavioral testing and biochemical assays and four for histological confirmation of the placement of the cut.

(d) Control operations. Ten rats served as operated controls. In these animals an incision was made in the scalp, holes were drilled through the skull, and a stainless steel insect pin was used to pierce the dura and cortex.

Response to Glucoprivic Challenges and Food Deprivation

Glucoprivation (2DG). Intraperitoneal (IP) injections of 2-deoxy-d-glucose (2DG) (650 mg/kg, 10% w/v in distilled water) were administered on Days 29 and 91 after surgery. Food intake during the first 6 hr after the injections was compared with the intake recorded on the previous day, during a comparable 6 hr period, following an injection of an equivalent volume of isotonic saline. The magnitude of the response to the drug of the operated animals was also compared to that of the control animals.

The experimental and control animals were also given a lower dose of 2DG (250 mg/kg, IP) accompanied by an injection of caffeine (25 mg/kg, of a 25 mg/ml solution of distilled water, IP). The test was conducted 105 days after surgery.

Both food and water were available ad lib during all tests. All injections were given at approximately 10 a.m. (3 hr after the beginning of the light period of the light/dark cycle). All testing took place in the animal's home cage.

Glucoprivation (Insulin). Intraperitoneal injections of 4 units of regular insulin (Iletin, Lilly, Co.) were given on the 52nd day after surgery. The food intake in the first six hr after the injection was compared with the intake recorded on the previous day during the same 6 hr period following an injection of a comparable volume of isotonic saline. The magnitude of the response to the drug in the experimental animals was also compared to the magnitude of the response in the control animals. Food and water were available ad lib during testing. The injections were given at 10 a.m.

Food deprivation. On the 39th and 100th day after surgery all animals were deprived of food for 18 hours. The food intake in the first 6 hr after deprivation was compared with the intake during a comparable six hr period on the previous day which was preceded by 18 hr of ad lib access to food. The intake of the experimental animals was also compared with the intake of the control animals. Water was available ad lib at all times.

Response to Hydrational Challenges

Water intake during food deprivation. On the 20th and 79th day after surgery water intake in the absence of food was recorded in the home cage for 24 hr. The results of this test were compared with the data from the preceding 24 hr period when food was present. The intake of the experimental animals was also compared with that of the controls.

Cellular dehydration (1M NaCl). On the 63rd day after surgery, all animals received IP injections of 5 ml of 1M NaCl solution. The water intake during the first 6 hr after the injections was compared with the intake during the same 6 hr period on the previous day after an IP injection of 5 ml of 0.15 M (isotonic) saline. The magnitude of the drinking response of the operated animals was also compared with that of the controls. Food and water were available ad lib during these tests. The injections were given at 10 a.m.

Biochemical Assays

At completion of behavioral testing, 115 days after surgery, the animals were killed by decapitation, the brains quickly dissected on ice into three brain regions (telencephalon, striatum, hypothalamus) and were assayed for regional amine concentrations. In each brain the concentrations of NE, DA and 5HT in striata and telencephalon and the concentrations of NE and 5HT in hypothalamus were determined by ion exchange chromatography as described by Barchas *et al.* [2] and modified by Halaris. Brain regions were homogenized in 15 ml ice cold 0.4 N perchloric acid with 0.25 ml 4% EDTA and 0.2 ml 2% ascorbic acid. After centrifugation, the supernatant was adjusted to pH 6.5 and passed through Amberlite (CG-50) columns. The amines were eluted from the column in 4 ml 1N HCl. Oxidations were performed according to Barchas *et al.* [2].

The telencephalon, striatum and hypothalamus were dissected by dividing the brain into three sections using coronal cuts at the rostral and caudal edges of the olfactory tubercles. The left and right striata were removed from the middle section by trimming away the cortex along the corpus callosum, removing the septum by cutting along the ventricles, and removing the amygdala by a horizontal cut. The cortical tissue surrounding the striatum, including the frontal lobes was combined with the remaining cortex of the other two sections to constitute the tissue of the telencephalon. The hypothalamus was dissected from the diencephalon by making a coronal cut just caudal to the mammillary bodies to remove the midbrain. The thalamus was removed and discarded from this section by cutting horizontally at the dorsal edge of the fornix on the anterior aspect of this section.

Histology

Fourteen rats with transections of the MFB were killed ten days after surgery with an overdose of Nembutal. The brains of these animals were perfused with formalin and frozen. Fifty μ sections were cut through the area of the knife cut and stained with cresyl violet. Of these animals four had PS cuts, six LMFB cuts and four MMFB cuts.

Correlations

Correlations were computed between regional amine concentrations and food or water intake in response to each of our experimental challenges, for the individual experimental groups as well as pooled data from all animals sustaining damage to the MFB. The absolute amount of food and water consumed during each test as well as the increase over baseline was used in the correlational analysis. Because a large number of correlations were computed, only coefficients which met a relatively stringent criterion of statistical reliability ($p<0.01$) are discussed.

RESULTS

Ad Lib Food and Water Intake

The effects of our cuts on 24 hr ad libitum food and water intake, and regional amine concentrations have been described in detail elsewhere [17]. Briefly, the 10 animals with PS cuts were aphagic and adipsic after surgery ($\bar{X}$ = 5.0 days, range 1 7). By Day 20 (the beginning of the tests described in the present paper) the daily food and water intake of the animals of this group was no longer significantly different from that of the control rats. The 12 rats with LMFB cuts were also aphagic and adipsic after surgery ($\bar{X}$ = 5.4, range 0 10). The seven animals from this group which were used in the present series of experiments were aphagic for only an average of 2.1 days (range, 1 5 days). By Day 20 (the beginning of the tests described in this report) the daily food and water intake of these animals was no longer significantly different from that of the controls. The 17 animals with MMFB cuts were hyperphagic after surgery and continued to overeat significantly ($p<0.01$) throughout the present series of experiments.

Regional Amine Concentrations (Fig. 1)

PS cuts resulted in large (greater than 50%) depletions of striatal DA and NE and telencephalic DA ($p<0.001$), moderate (approximately 30%) depletions of telencephalic NE and 5HT and striatal 5HT ($p<0.01$), and little or no change in hypothalamic amines. LMFB cuts resulted in large (greater than 50%) depletions of striatal DA ($p<0.001$), moderate (approximately 30%) changes in striatal NE and 5HT and telencephalic DA and NE ($p<0.01$), and little or no change in hypothalamic amines or telencephalic 5HT. MMFB cuts resulted in moderate decreases in telencephalic NE, DA, and 5HT ($p<0.001$), somewhat smaller depletions of striatal NE, DA, and 5HT ($p<0.01$) and little or no change in hypothalamic amines. (For a detailed discussion of these data see [17]).

Histology (Fig. 2)

Analysis of cresyl violet stained sections of fourteen brains confirmed that the knife cuts had been placed at the intended coordinates as described in the methods section of this paper. Consistent placements of similar knife cuts have been reported in several earlier papers from this laboratory [1,12,17].

The PS cuts (Fig. 2A) extended bilaterally along the lateral border of the diencephalon just medial to the internal capsule, from AP = 6.2, to AP = 4.2 and from the subthalamus to the base of the brain. The LMFB cuts (Fig. 2B) lay in a coronal plane just anterior to the

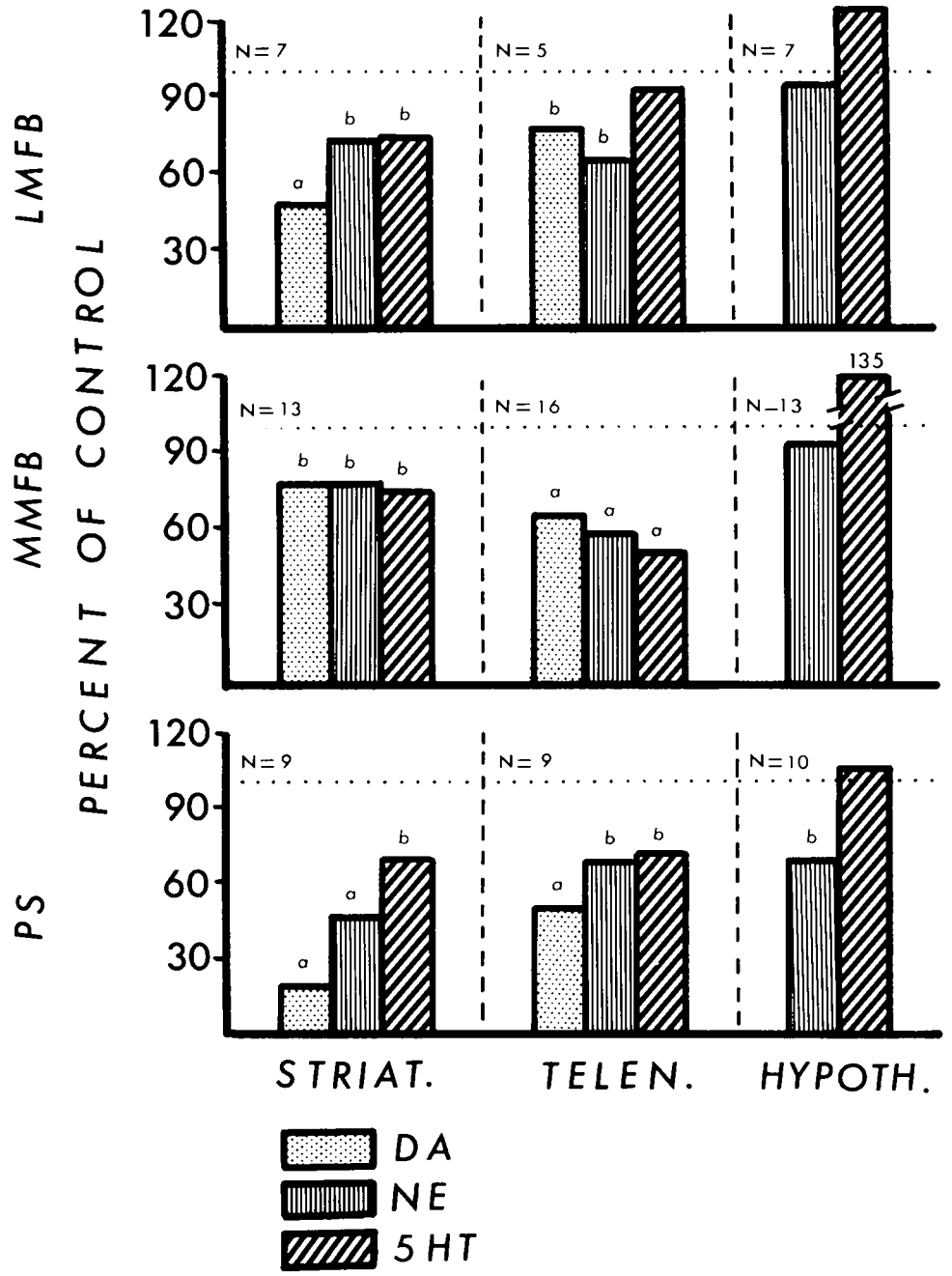

FIG. 1. Percent of control concentrations of brain amines for animals with parasagittal cuts (PS) along the lateral border of the diencephalon, coronal cuts across the medial fibers of the MFB (MMFB), or coronal cuts across the lateral fibers of the MFB (LMFB). Striatal concentrations in control brain were 372 ± 65 ng/g NE, 9674 ± 2029 ng/g DA, and 706 ± 112 ng/g 5HT. Hypothalamic concentrations in control brain were 2092 ± 246 ng/g NE, and 1428 ± 508 ng/g 5HT. Telencephalic concentrations in control brain were 478 ± 43 ng/g NE, 785 ± 63 ng/g DA and 613 ± 100 ng/g 5HT. [a]Significantly different from control levels ($p<0.001$). [b]Significantly different from control levels ($p<0.01$).

mammillary bodies at AP = 4.6 and transected fibers of the MFB that ascend or descend in the medial segments of the internal capsule. The MMFB cut (Fig. 2C) lay in a coronal plane just anterior to the mammillary bodies and transected the medial fibers of the MFB as they course through the lateral hypothalamus in an area bounded by the internal capsule, medial lemniscus, fornix and base of the brain.

Glucoprivation (2DG) (Fig. 3).

PS cuts. Animals with PS cuts showed an impaired response to 2DG, 29 as well as 91 days after surgery. Animals with PS cuts ate reliably ($p< 0.05$) more food after 2DG than they had on the saline control test but the

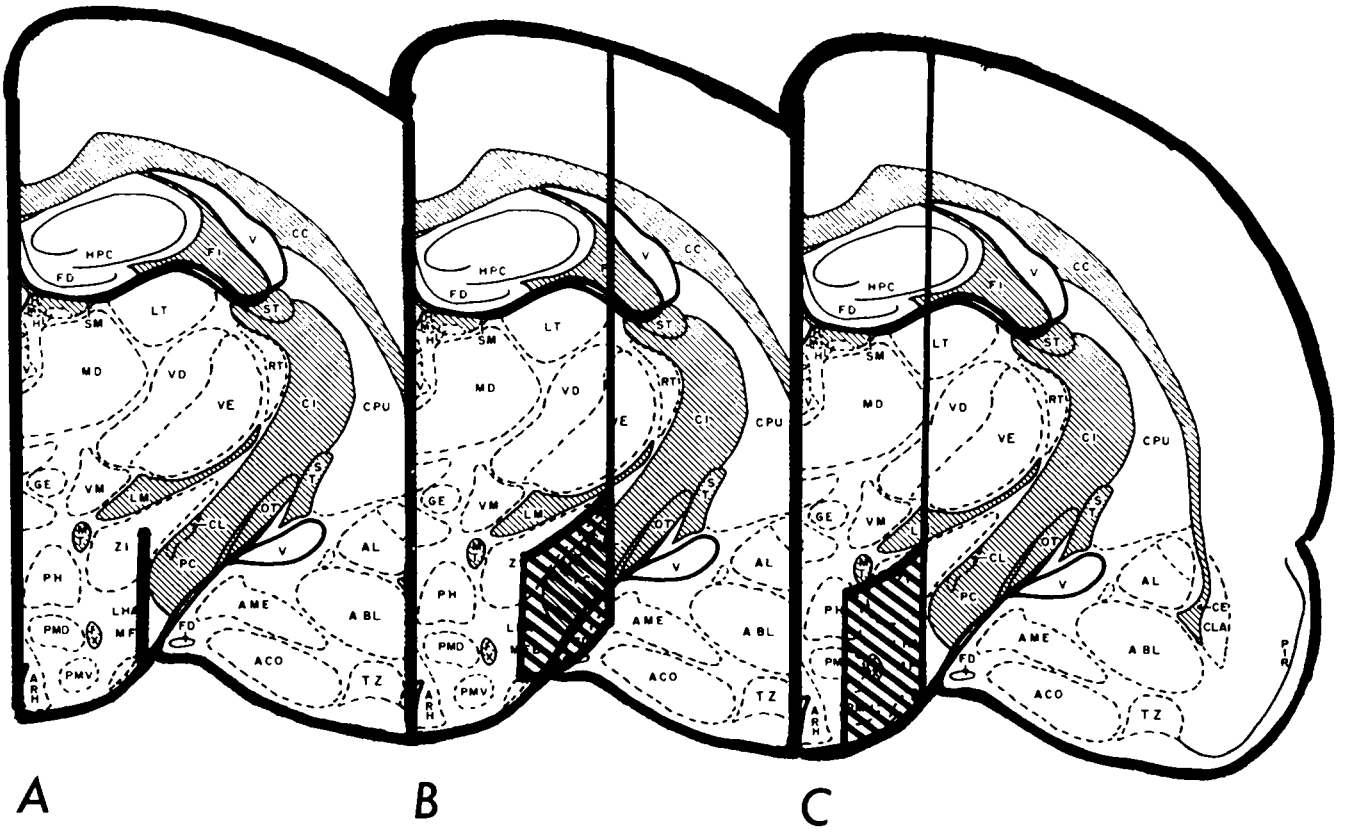

FIG. 2. Schematic diagram of (A) parasagittal cuts along the lateral border of the diencephalon (PS) extending from AP = 6.2 to 4.2, (B) coronal cuts across the lateral fibers of the MFB (LMFB) at AP = 4.6 and (C) coronal cuts across the medial fibers of the MFB (MMFB) at AP = 4.6, according to the de Groot atlas of the rat brain [7].

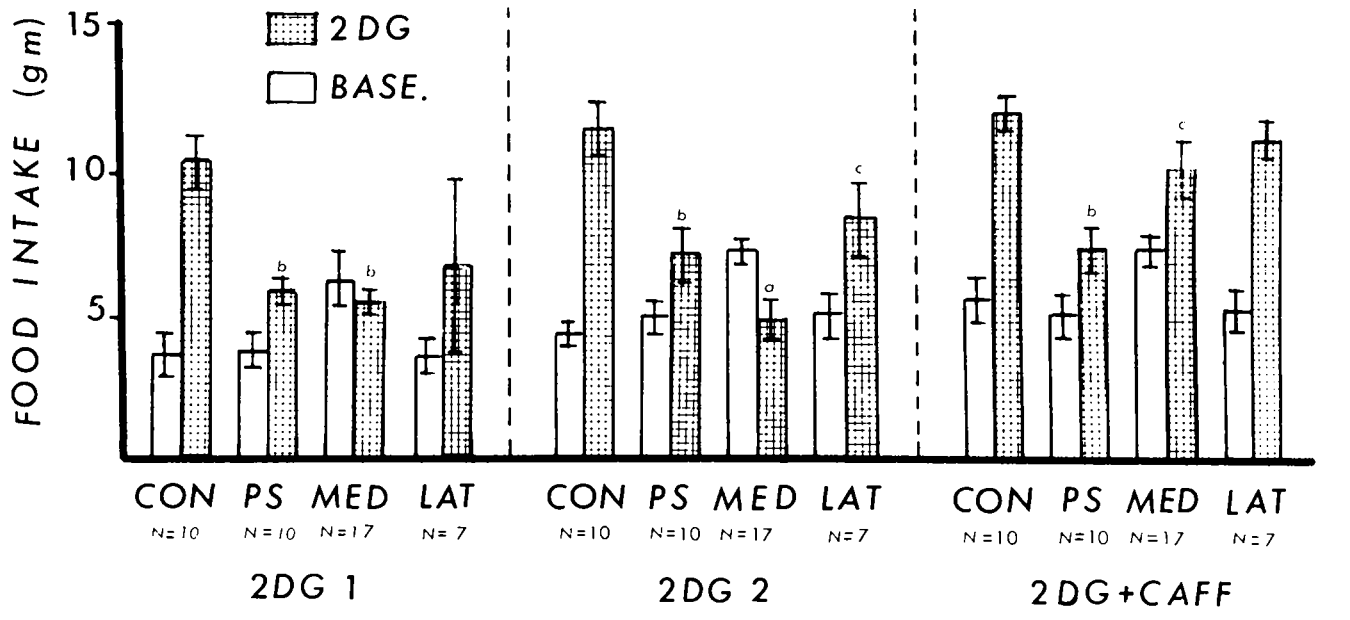

FIG. 3. Food intake during the first six hr after injections of isotonic saline (BASE) or 2-deoxy-d-glucose (2DG) (650 mg/kg) on Day 29 (2DG 1) and Day 91 (2DG 2) or in response to 2-deoxy-d-glucose (250 mg/kg) plus caffeine (25 mg/kg) on Day 105 (2DG + CAFF) after surgery for animals with parasagittal knife cuts (PS), medial MFB cuts (MED), lateral MFB cuts (LAT), or controls (CON). [a]Significantly different from control intake ($p<0.001$). [b]Significantly different from control intake ($p<0.01$). [c]Significantly different from control intake ($p<0.05$).

magnitude of the response was significantly smaller than that seen in the controls on both test days ($p<0.01$, $p<0.003$, respectively).

LMFB cuts. Animals with LMFB cuts did not increase their food intake significantly ($p>0.20$) in response to 2DG when tested 29 days after surgery. Although these animals did reliably ($p<0.05$) increase intake after 2DG when tested 91 days after surgery, the magnitude of the response was significantly ($p<0.05$) impaired with respect to controls.

MMFB cuts. Animals with MMFB cuts did not respond to 2DG when tested 29 days after surgery. There was no significant difference between their intake after the drug and the intake on the saline control test. The control group increased intake reliably ($p<0.001$) on the drug test. When retested 91 days after surgery, animals with MMFB cuts ate reliably ($p<0.01$) less after 2DG than they did on the preceding control test. The control animals showed a significant ($p<0.01$) increase. Although the operated animals were hyperphagic with respect to the controls at the time of the test, the total food intake in response to 2DG, of the rats with MMFB cuts, was significantly smaller than that of the controls, on both test days ($p<0.01$, $p<0.001$, respectively).

Glucoprivation (2DG+caffeine) (Fig. 3)

PS cuts. The food intake of animals with PS cuts after injections of a low dose of 2DG, accompanied by caffeine was significantly ($p<0.05$) elevated from baseline but was significantly ($p<0.01$) smaller than the intake of the controls. The feeding response to 2DG plus caffeine was not significantly ($p>0.25$) different from the response to 2DG alone.

LMFB cuts. The food intake of animals with LMFB cuts was significantly ($p<0.001$) increased with respect to the control test baseline after 2DG accompanied by caffeine. The intake of the animals with LMFB cuts was not significantly different on this test from that of the controls ($p>0.05$).

MMFB cuts. Rats with MMFB cuts increased their food intake reliably ($p<0.05$) in comparison to baseline after the low dose of 2DG combined with caffeine. The intake of the experimental group was reliably smaller ($p<0.05$) than that of the control group. The feeding response to 2DG plus caffeine was significantly ($p>0.001$) larger than the response after 2DG alone.

Glucoprivation (Insulin) (Fig. 4)

PS cuts. Animals with PS cuts did not reliably respond to 4 units of insulin when tested 52 days after surgery. The intake of the operated animals was significantly smaller ($p<0.01$) than that of the controls and was not significantly ($p>0.05$) elevated from baseline levels established on the preceeding control test.

LMFB cuts. Animals with LMFB cuts responded to insulin by eating significantly ($p<0.01$) more food than they had on the saline control test. Their intake was not significantly ($p>0.05$) different from intake of controls.

MMFB cuts. Animals with MMFB cuts significantly ($p<0.001$) increased their food intake after insulin. The magnitude of their response was not significantly ($p>0.05$) different from intake of controls.

Food Deprivation (Fig. 5)

PS cuts. Following 18 hr of food deprivation, the food

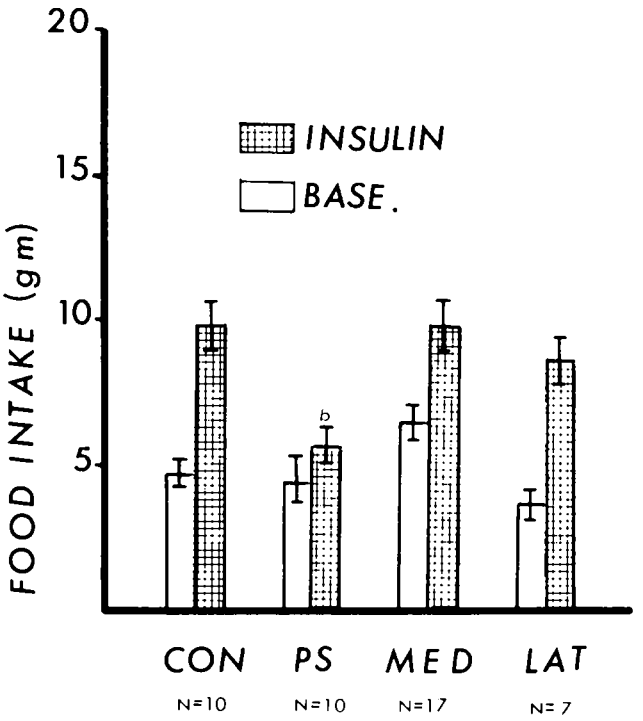

FIG. 4. Food intake during the first 6 hr after injections of isotonic saline (BASE) or insulin (4 units) on Day 52 after surgery for animals with parasagittal knife cuts (PS), medial MFB cuts (MED), lateral MFB cuts (LAT), or controls (CON). [b]Significantly different from control intake ($p<0.01$).

intake of animals with PS cuts was not significantly ($p>0.10$) different from the intake of the controls, 39 and 100 days after surgery.

LMFB cuts. Animals with LMFB cuts, ate significantly ($p<0.05$) more after 18 hr of food deprivation than they did during the preceding control test but the magnitude of the increase was reliably ($p<0.001$) smaller than that of the controls on both test days.

MMFB cuts. Thirty nine days after surgery the animals with MMFB cuts ate significantly ($p<0.001$) less than the controls after food deprivation. The operated animals increased their intake 3.4 g over the baseline established without prior deprivation whereas the controls increased their intake by 12.0 g. The deficit persisted when the animals were retested 100 days after surgery. The impairment is particularly interesting in view of the fact that the operated animals were hyperphagic ($p<0.01$) with respect to controls in 24 hr ad lib intake, and ate reliably more than the controls ($p<0.01$) on the preceding control test.

Water Intake in the Absence of Food (Fig. 6)

PS cuts. When tested in the absence of food, animals with PS cuts drank significantly less than the controls both 20 and 63 days after surgery ($p<0.001$, $p<0.01$, respectively).

LMFB cuts. In the absence of food, the water intake of animals with LMFB cuts, was significantly ($p<0.01$) smaller than that of the controls 20 days after surgery. The effect was not apparent when this experiment was repeated 79 days after surgery ($p>0.05$).

MMFB cuts. Animals with MMFB cuts drank quantities of water during food deprivation that were not significantly

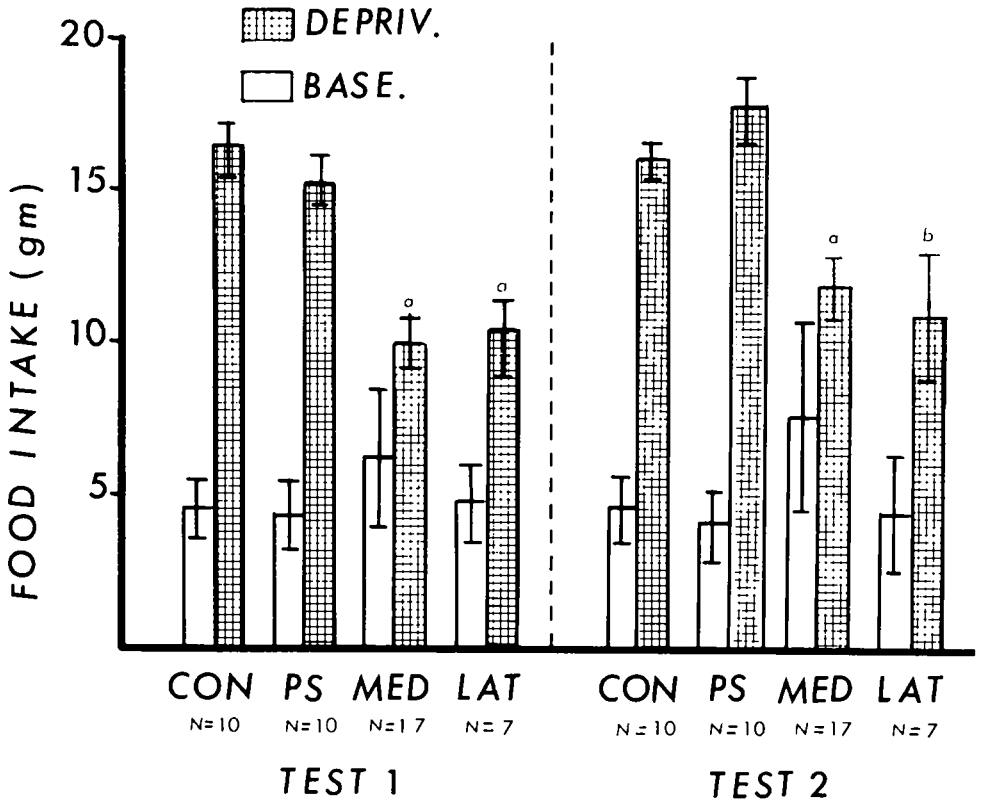

FIG. 5. Food intake during the first six hr after 18 hr ad lib access to food (BASE) or after 18 hr of food deprivation (DEPRIV), 39 days (TEST 1) and 100 days (TEST 2) after surgery, for animals with parasagittal cuts (PS), medial MFB cuts (MED), lateral MFB cuts (LAT) or control (CON). [a]Significantly different from control intake ($p<0.001$). [b]Significantly different from control intake ($p<0.01$).

different from those consumed by the controls ($p>1.0$) both 20 and 79 days after surgery.

Cellular Dehydration (1M NaCl) (Fig. 7)

PS cuts. When injected with hypertonic saline on day 63, animals with PS cuts drank significantly less than controls ($p<0.001$). The water intake of the experimental animals was reliably ($p<0.05$) greater than their own control baseline but the increase was only half as large as that seen in the controls.

LMFB cuts. The response of animals with LMFB cuts to hypertonic saline was statistically reliable ($p<0.05$) but also significantly ($p<0.01$) smaller than the effect seen in the controls.

MMFB cuts. Animals with MMFB cuts increased their water intake ($p<0.05$) after injections of hypertonic saline, but drank significantly ($p<0.01$) less than the controls. This effect is particularly interesting in view of the fact that the 24 hr water intake of the experimental animals was reliably ($p<0.05$) greater under ad lib conditions. The water intake during the 6 hr test used as a baseline for these experiments was not reliably different ($p>0.05$) from that of the controls.

Correlations

Because of the large number of correlations computed, only coefficients that exceeded the 0.01 probability level for pairwise comparisons are included in the following discussion. A correlational analysis of pooled data from all experimental animals revealed several significant ($p<0.01$) correlations between responses to regulatory challenges and regional amine concentrations which support trends (large correlations which did not meet our stringent statistical criterion) observed in individual groups.

2DG. Analysis of the pooled data from all animals with damage of the MFB, revealed reliable positive correlations ($r = .47$, $r = .45$, respectively) between telencephalic NE and the magnitude of the increase in intake over baseline in response to 2DG on Days 29 and 91. There were no significant correlations between the magnitude of the response to 2DG and the duration of aphagia after surgery.

2DG+caffeine. The magnitude of the feeding response to 2DG plus caffeine was negatively correlated ($r = -.58$, $r = -.64$, respectively) with striatal 5HT concentrations in rats with LMFB or PS cuts. An analysis of pooled data from all experimental animals did not provide further support for this finding. There was no significant correlation between the magnitude of the response to 2DG plus caffeine and the duration of aphagia after surgery or response to 2DG alone.

Insulin. Our analysis of pooled data from all animals with transections of the MFB, indicated that the magnitude of the feeding response to insulin was positively correlated with the concentration of striatal DA ($r = .61$). The response to insulin was also negatively correlated with the duration of aphagia after surgery ($r = -.49$), indicating that

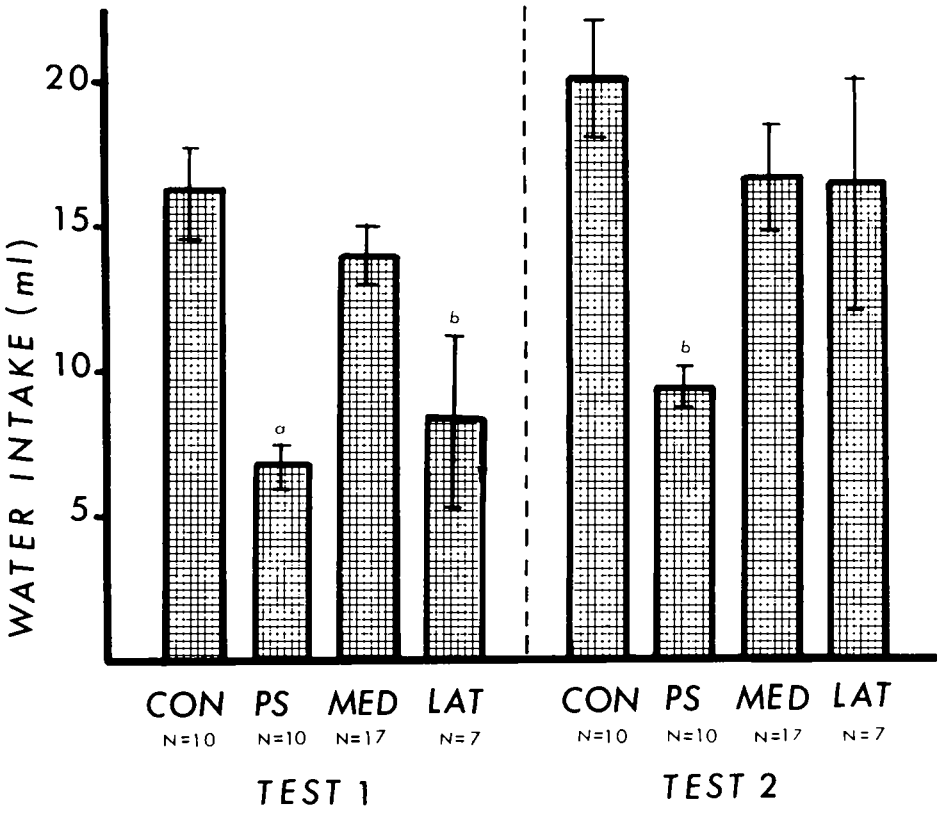

FIG. 6. Twenty-four hr water intake in the absence of food on Day 20 (TEST 1) and Day 79 (TEST 2) after surgery for animals with parasagittal cuts (PS), medial MFB cuts (MED), lateral MFB cuts (LAT), or controls (CON). [a]Significantly different from control intake ($p<0.001$). [b]Significantly different from control intake ($p<0.01$).

the severity of the deficit is positively correlated with severity of the aphagia.

Food deprivation. Food intake following deprivation was negatively correlated with telencephalic DA concentrations in rats with PS cuts on both test days (r = -.74, r = -.76, respectively). Our analysis of pooled data revealed several additional correlations. Food intake on both deprivation tests was negatively correlated with the concentrations of striatal DA (r = -.57, r = -.47); telencephalic DA (r = -.62, r = -.54) and hypothalamic NE (r = -.43, r = -.54). There was a positive correlation between the magnitude of the response to food deprivation and the duration of aphagia on both tests (r = .71 and r = .68), indicating that the severity of the deficit in response to food deprivation is negatively correlated with the severity of the aphagia. Determination of partial correlations suggests that the correlations observed between DA and the response to food deprivation may be an artifact of the relationship observed between aphagia and DA or between aphagia and the response to food deprivation.

Water intake in the absence of food. Our analysis of data from individual experimental groups indicated that on both tests water intake in the absence of food correlated positively with hypothalamic NE (r = .76, r = .72) in rats with MMFB cuts. The analysis of pooled data revealed a significant correlation between water intake on both tests and striatal DA (r = .49, r = .47, respectively). There was no significant correlation ($p>0.1$) between water intake in the absence of food and the duration of aphagia.

1M NaCl. Our analysis of individual group data or pooled data revealed no significant relationships between water intake after 1M NaCl and regional amine concentrations. There was also no significant correlation between the response to hypertonic saline and the duration of aphagia and adipsia after surgery. In control animals water intake after 1M NaCl was positively correlated (r = .80) with telencephalic concentrations of NE.

DISCUSSION

We [17] have reported earlier that transections of 3 components of the MFB produced different patterns of regional amine depletions which correlated with changes in food and water intake. When data from all experimental groups were pooled, significant positive correlations were obtained between daily food and water intake and striatal and telencephalic dopamine; similar correlations were obtained in the individual groups. These data are congruent with the hypothesis that normal feeding behavior requires intact dopaminergic projections to the striatum and telencephalon [15,20,22].

The results of the present experiments do not support the related hypothesis that the deficits in response to glucoprivic and hydrational challenges that are characteristic of rats which have recovered voluntary ingestive behavior after LH lesions or intracranial 6OHDA treatments [15,23] are due to incomplete recovery of the same pathways. Our observations indicate, instead, that the syndrome of persisting deficits may be the result of

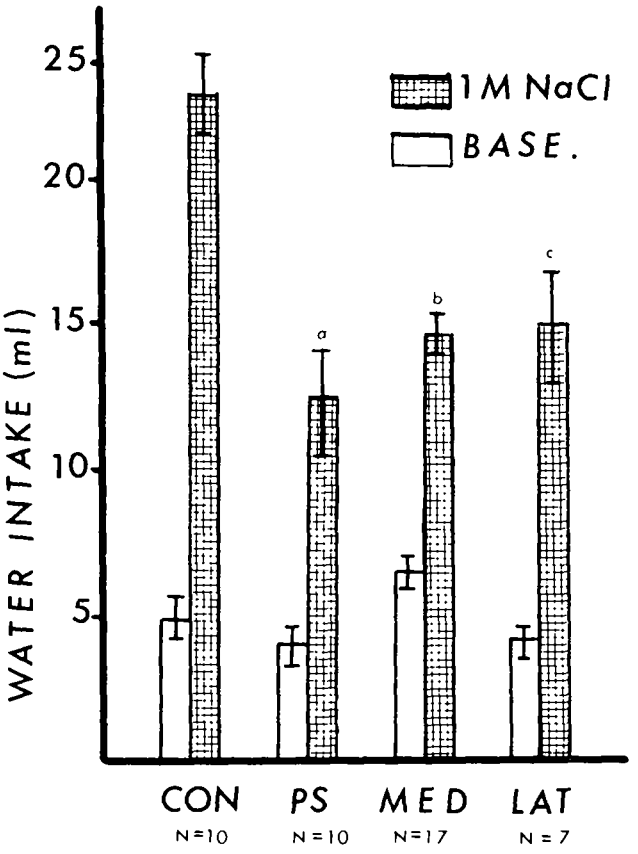

FIG. 7. Water intake during the first 6 hr after injections of isotonic saline (BASE) or hypertonic saline (1 M NaCl), 63 days after surgery for animals with parasagittal cuts (PS), medial MFB cuts (MED), lateral MFB cuts (LAT) or controls (CON). [a]Significantly different from control intake ($p<0.001$). [b]Significantly different from control intake ($p<0.01$). [c]Significantly different from control intake ($p<0.05$).

functional impairments in several anatomically as well as neurochemically distinct neural pathways and that few, if any, of the behavioral deficits are the result of a persisting dysfunction of the pathways which are responsible for the initial aphagia and adipsia. These conclusions are supported by several considerations.

In good agreement with observations from this laboratory [1,10], rats with PS cuts along the lateral border of the hypothalamus failed to respond normally to experimental glucoprivic or hydrational challenges and drank little or no water in the absence of food. This pattern of persisting deficits is identical to that seen after lateral hypothalamic lesions. That individual components of this syndrome may be independent of each other and unrelated to the initial aphagia and adipsia that is such a prominent, if transient, feature in rats with LH lesions or PS cuts, is suggested by our findings that (a) rats with MMFB cuts which were hyperphagic after surgery, showed many of the same persisting deficits (notably a lack of response to 2DG, impaired reactions to food deprivation, and reduced drinking after an osmotic challenge). (b) Rats with LMFB cuts were aphagic and adipsic after surgery but displayed a pattern of persisting deficit that was different, in several important respects, from that seen in rats with PS cuts. After recovery of voluntary intake, rats with LMFB cuts responded poorly to 2DG but ate promptly in response to insulin. Pretreatment with a CNS stimulant increased the response to 2DG in these animals. Rats with PS cuts, on the other hand, failed to respond normally to insulin, 2DG, and to the combined 2DG plus caffeine treatments. Rats with LMFB cuts (but not animals with PS cuts) failed to respond normally to food deprivation suggesting that the effects of the two cuts may differ qualitatively. (c) The persisting deficits seen in rats with LMFB cuts (which produced aphagia and adipsia) were similar to those seen in animals that were hyperphagic after MMFB cuts (both responded poorly, if at all to 2DG but ate normally after insulin, failed to eat normal quantities of food after a period of deprivation, and drank little in response to an osmotic challenge). Only the test of drinking in the absence of food and the initial effect on ad lib food intake indicated a signficant difference between the LMFB and MMFB group. (d) Only one of the persisting deficits (the feeding response to insulin) was positively correlated with the duration of the aphagia and adipsia.

The different behavioral effects of our cuts are particularly interesting when examined in the context of their differential effects on regional amine concentrations. By far the most severe effects on striatal DA and NE were seen in animals with PS cuts. Rats with LMFB cuts sustained very much smaller depletions of striatal catecholamines, and rats with MMFB cuts (which produce many of the persisting deficits although the animals were hyperphagic) displayed only small effects on striatal NE and DA. Striatal 5HT was reduced only slightly in all three groups. There were only small differences in the effectiveness of our cuts in depleting telencephalic catecholamine concentrations, and only the PS cuts depleted hypothalamic NE reliably.

The results of a correlational analysis of the behavioral and biochemical effects of these cuts indicate that the persisting deficits do not appear to be related to a dysfunction in a common biochemical mechanism such as the nigrostriatal projections. The concentration of striatal dopamine was reliably correlated only with the magnitude of the feeding response to insulin, and the amount of water consumed during food deprivation. This is congruent with reports of impaired responsiveness to insulin after intracisternal as well as intraventricular injections of 6OHDA [5,22] and reports of significant correlations between striatal DA and the duration of aphagia and adipsia after intrahypothalamic injections of 6OHDA [15] or electrolytic lesions in the LH [20].

In view of these correlations, it is interesting that neither the feeding response to 2DG nor the drinking response to NaCl was significantly correlated with striatal DA. The former was related to telencephalic NE, the latter was not reliably correlated with any of the regional amine measures obtained in this investigation. These observations are in good agreement with reports from other laboratories. Deficits in the response to 2DG have been reported after intraventricular as well as intracerebral injections of 6OHDA which depleted telencephalic NE as well as striatal NE and DA [3,15,22]. That these effects are probably related to the effects of the neurotoxin on NE neurons is suggested by several observations. Impaired feeding responses to 2DG have been reported after lesions of the preoptic area [3] which are not in the trajectory of the nigrostriatal pathway. The peculiar combination of impaired or absent feeding responses to 2DG and intact reactions to insulin has been

seen (a) in rats with knife cuts in the tegmentum [11] that depleted hypothalamic as well as forebrain NE [11] but were far caudal to the cells of origin of striatal or telencephalic DA projections (b) in rats with ventromedial hypothalamic lesions [14] that should not have interferred with dopaminergic projections to the striatum and telencephalon and (c) lesions in the zona incerta [28] which did not result in significant striatal DA depletions [27].

That the effects of our cuts on water intake after hypertonic saline did not correlate with amine concentrations in any part of the brain is also congruent with reports from other laboratories. Several investigators [5,22] have reported that intracisternal or intraventricular injections of 60HDA produced various deficits in ingestive behavior but failed to reduce the drinking response to hypertonic saline. Others [15] have reported impairments in this test after intrahypothalamic injections of 60HDA but failed to establish significant correlations between this behavioral effect and brain amine depletions. It is, moreover, possible that nonspecific tissue damage may have contributed significantly to their effects after intracerebral injections of 60HDA [6].

The magnitude of the drinking response to NaCl was reliably correlated with telencephalic NE in the control animals. In spite of our strict statistical criterion, this might well be artifactual but it is noteworthy that a significant correlation between the response to NaCl and hypothalamic NE has been observed in another investigation from our laboratory [1].

Two additional features of our results require comment. It is generally assumed that 2DG and insulin elicit feeding because both agents decrease intracellular glucose utilization and that this is monitored by a peripheral or central glucostat which translate changes in the intracellular availability of glucose into feeding or satiety signals. A number of recent experimental observations have suggested that this may be too simplistic a view. Several brain lesions, including damage to the zona incerta [28], ventromedial hypothalamus [14] or midbrain tegmentum [1] abolish or severely impair feeding responses to 2DG without reducing the response to insulin. In the present experiment we observed that the response to 2DG was positively correlated with NE concentrations in the telencephalon, whereas the response to insulin was positively correlated with the concentration of DA in the striatum. Furthermore, the behavioral responses to these two experimental glucoprivic challenges were not correlated with each other. These observations indicate that the central pathways which mediate the response to the two types of glucoprivic challenges are at least to some extent different. Feeding response to 2DG is also delayed or reduced by vagotomy which does not impair the feeding response to insulin [4,8,19]. It is thus possible that the CNS lesions which produce a similar pattern of effects do so, at least in part, because they interrupt secondary or tertiary vagal projections to the diencephalon.

Lastly, we would like to comment on the results of our test of feeding responses to 2DG combined with caffeine. Stricker and Zigmond [24] have recently suggested that aphagia and adipsia and persisting deficits in response to glucoprivic and hydrational challenges that are seen after LH lesions or intracranial injections of 60HDA may reflect impaired arousal rather than a dysfunction of mechanisms specifically related to the regulation of food and water intake. In support of this interpretation, Stricker and Zigmond cite instances where the addition of arousing stimuli facilitated responding to glucoprivic challenges in rats which had recovered voluntary ingestive behavior after intraventricular injections of 60HDA.

We repeated one of these tests in our animals and found that the addition of caffeine restored normal feeding responses in rats with LMFB cuts but had only smaller effects in rats with MMFB or PS cuts. This pattern of effects suggests that the different cuts may have interferred with the feeding response to 2DG for quite different reasons. Although our cuts did not produce the severe impairments in arousal typical of 60HDA treated animals, it would appear that reduced endogenous activation may have contributed to the impaired response to 2DG observed in animals with LMFB cuts. Rats with PS cuts and MMFB cuts, on the other hand, showed only slight improvement after the caffeine treatment and it seems possible that some interference with mechanisms not concerned with general arousal is responsible for the impaired response to 2DG in these groups.

A closer look at our correlation matrix indicates that the response to 2DG and the response to 2DG plus caffeine may, in fact, be mediated by different mechanisms. There was no reliable correlation ($r = .22$, $p > 0.20$) between the magnitude of the feeding response to 2DG and 2DG plus caffeine; the response to 2DG plus caffeine but not the response to 2DG was reliably ($r = .40$, $p < 0.01$) correlated with the magnitude of the feeding response to insulin; and the feeding response to 2DG was reliably correlated with telencephalic NE whereas the response to 2DG plus caffeine was correlated with striatal 5HT. In the context of Stricker and Zigmond's [23] arousal hypothesis, it is particularly interesting that the response to 2DG plus caffeine was not reliably correlated ($r = .05$, $p > 0.70$) with the duration of aphagia or with concentrations of DA ($r = .25$, $p > 0.20$, $r = .02$, $p > 0.90$) or NE ($r = .30$, $p > 0.10$, $r = .01$, $p > 0.90$) in the striatum or telencephalon.

In summary, our data suggest that the regulatory deficits which occur after damage to pathways in the medial forebrain bundle and lateral hypothalamic area may not be the result of depletions of dopamine from the striatum or telencephalon or norepinephrine from the hypothalamus. Furthermore, these deficits appear to be unrelated to the biochemical changes which are responsible for the initial period of aphagia and adipsia. Our data indicate that individual deficits may be mediated by biochemically distinct pathways.

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