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Reduced Forebrain Norepinephrine in Rhesus Monkeys Made Obese by Hypothalamic Lesions[1]

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KEMNITZ, J. W., G. W. KRAEMER AND G. R. BREESE. *Reduced forebrain norepinephrine in rhesus monkeys made obese by hypothalamic lesions.* PHARMAC. BIOCHEM. BEHAV. 13(3) 461–465, 1980.—Young adult male rhesus monkeys received bilateral lesions in the anteromedial lateral hypothalamus. During the year of observation following surgery the animals were hyperphagic and became markedly obese. The mean increase in body weight averaged 66% more for the experimental animals than for the controls. Analyses of catecholamine concentrations in 12 brain regions at the time of sacrifice revealed consistently lower concentrations of norepinephrine (averaging 41% of control) in the frontal cortex of the obese animals as well as a more variable decrease in norepinephrine in the caudate and striatum. There was no consistent effect of the lesions on dopamine in any of the regions studied. There was no obvious relationship between regional catecholamine levels and weight gain within the experimental group.

Hypothalamic lesions Obesity *Macaca mulatta* Norepinephrine Dopamine Body weight Catecholamines

THE relationship between lesion-induced depletions of brain catecholamines (CA) and obesity is not well defined, particularly for primates. In the rat, lesions in the hypothalamus and midbrain that are effective in producing obesity reliably deplete forebrain norepinephrine (NE) [1, 5, 8, 10, 19], although the magnitude of the obesity and severity of NE depletion are not always well correlated [10,12]. In primates, it has been clearly established that certain hypothalamic lesions are effective in producing obesity [2, 6, 11, 17, 20, 23], but correlative information concerning brain CA has not been reported. However, lesions of the locus coeruleus (LC) in *Macaca arctoides* produced marked obesity which was highly correlated with reduced cortical levels of 3-methoxy-4-hydroxyphenylglycol (MHPG), a product of NE degradation [24,25]. We report here two new findings: (1) lesions of the anteromedial lateral hypothalamus of rhesus macaques produced obesity and (2) forebrain NE was depleted in these obese animals.

METHOD

Five male *Macaca mulatta*, 6–10 years of age, were used in this study. They were individually housed in metabolism cages in a light- and temperature-controlled room (13L:11D, 21°C) which contained other similarly housed monkeys. Fresh food (Purina Monkey Chow) was provided twice daily in amounts that insured maximal levels of voluntary intake except during occasional periods of restriction for experimental purposes and tap water was available ad lib. Body weights were recorded at least weekly.

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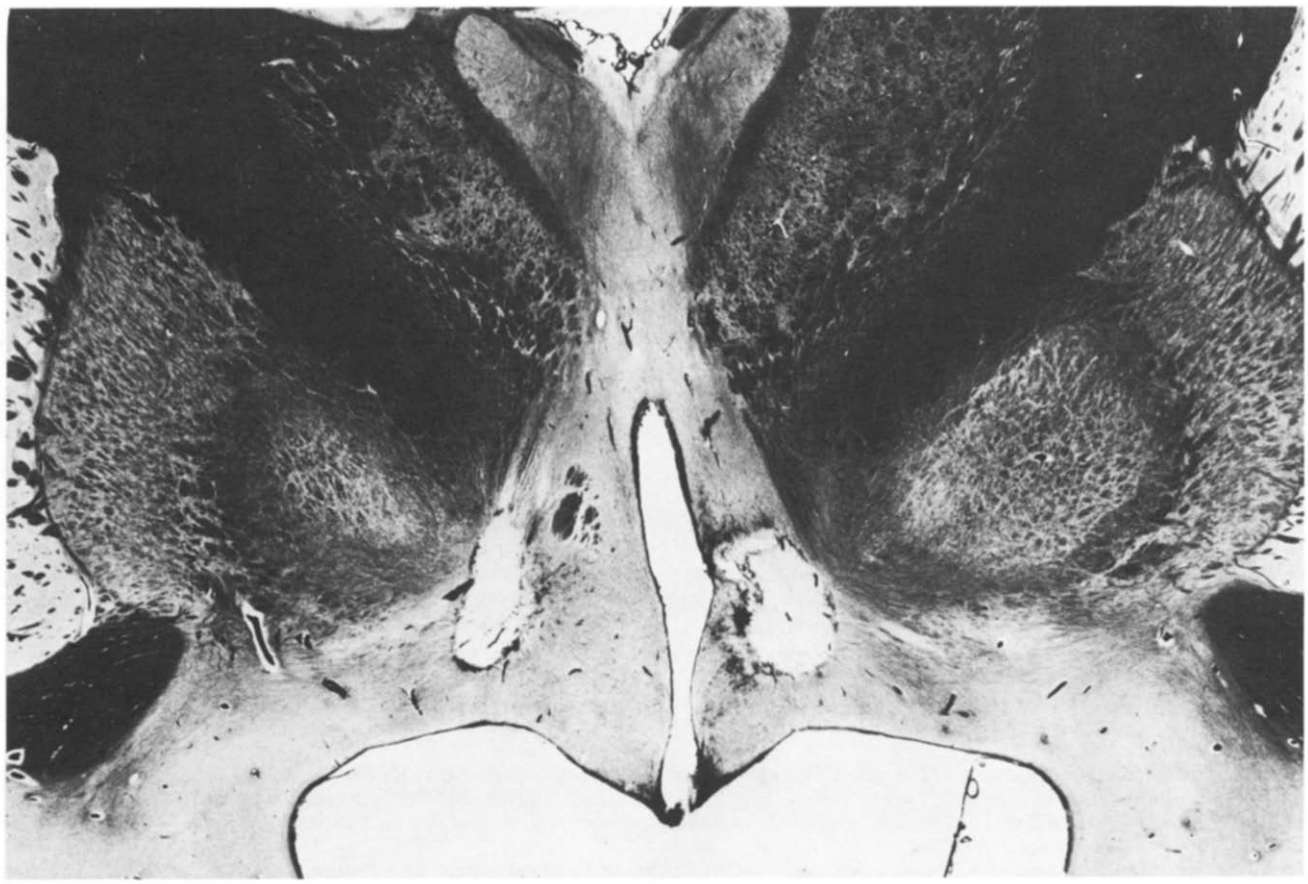
TABLE 1

BODY WEIGHTS AND REGIONAL CATECHOLAMINE CONCENTRATIONS

	Experimentals				Controls		
	1572	G67	1720	$\bar{X} \pm SD$	1574	1575	$\bar{X} \pm SD$
Body weight (kg)							
Presurgical	8.90	9.38	10.32	9.53 ± 0.59	9.38	12.01	10.70 ± 1.32
Maximal	18.15	18.14	15.58	17.29 ± 1.21	10.78	14.19	12.48 ± 1.70
(weeks after surgery)	(54)	(75)	(53)		(39)	(70)	
Terminal	16.42	18.14	14.79	16.45 ± 1.37	8.36	13.78	11.07 ± 2.71
(weeks after surgery)	(76)	(75)	(75)		(72)	(72)	
Norepinephrine (ng/g)							
Orbital cortex*	79/83	59/ 56	62/53†	62 ± 7	171/168	158/134†	155 ± 4
Dorsal cortex*	58/63	40/108	58/39†		117/154	142/196†	
Caudate + striatum†	30	4	11	15 ± 11	46	34	40 ± 6
Cerebellum + brainstem†	231	228	299	253 ± 33	218	238	228 ± 10
Dopamine (ng/g)							
Orbital + dorsal cortex†	63	43	137	81 ± 40	87	81	84 ± 3
Caudate + striatum†	7,646	5,080	10,644	7,790 ± 2,274	7,931	9,802	8,866 ± 936
Cerebellum + brainstem†	29	25	39	31 ± 6	20	26	23 ± 3

*Left hemisphere/right hemisphere.
†Combined value for two regions and both hemispheres.



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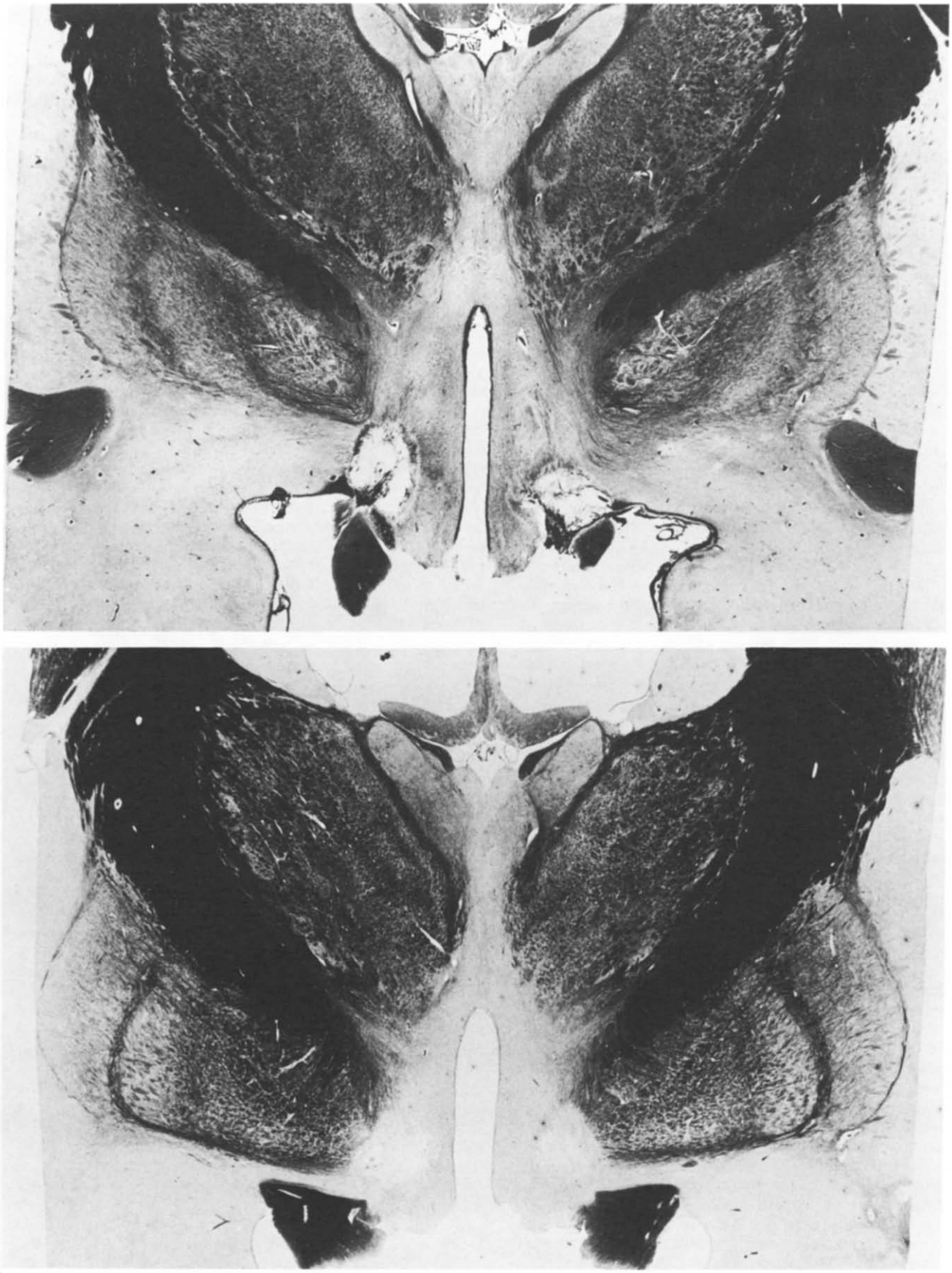

FIG. 1. Photomicrographs of frontal sections through the hypothalamus at approximately the maximal extent of the lesions for animals 1572 (A), 1720 (B), and G67 (C).

Radiofrequency lesions were produced bilaterally in three of the animals (Grass Model LM3, Intensity=80, Duration 20 sec) and sham lesions (electrode lowered to lesion site but no heat generated) were produced in the other two animals. Only one of the experimental animals exhibited a clear change in body weight, and so the lesions of all three experimental animals were enlarged approximately 15 weeks after the first surgery (Radionics RFG-4, 70°C for 1.0 min). The electrodes were placed using a radiographic procedure in which the lateral ventricles were visualized using pneumoencephalography [16], and then 0.1 cc megulamine iothalamate (Conray) was injected adjacent to the foramen of Monro to facilitate visualization of the third ventricle. The target site was 1.0 mm caudal from the rostral extreme of the third ventricle, 2.5–3.0 mm bilateral from midline, and 1.5 mm ventral to a line connecting the tip of the anterior clinoid process and the cerebral aqueduct.

Approximately 12 months after the lesions were enlarged, the animals were sedated with ketamine (10 mg/kg, IM) and 15 min later deeply anesthetized with thiamylal sodium (25 mg/kg, IV). The brain was removed, placed on a chilled platform and quickly dissected. Parallel frontal sections were made approximately 5 mm anterior to the optic chiasm and just caudal to the mammillary bodies. The tissue between these sections was placed in 10% formal saline for subsequent light microscopic analysis. The remaining brain parts were divided by midsagittal section and further dissected into the following parts: caudate nucleus, rest of the corpus striatum, dorsal cortex, orbital cortex, cerebellum, and brain stem. These 12 brain pieces per animal were individually placed in scintillation vials and dipped in liquid nitrogen. As soon as the nitrogen evaporated, the vials were capped and kept on dry ice until each sample was analyzed for NE and dopamine (DA) content [4]. No more than three minutes elapsed between interruption of blood supply to the brain and freezing of the samples. The portion of the brain containing the hypothalamus was fixed in 10% formal saline and later transferred to 30% sucrose-10% formal saline before taking serial frontal frozen sections (50 μ thick). The first section in every series of three sections was stained with cresyl violet and the second in each series was stained according to Heidenhain's hematoxylin procedure. The stained sections were examined microscopically to determine the location and extent of the lesions.

RESULTS

The three experimental animals were hyperphagic and became markedly obese. Weight gains were approximately proportional to food intake. Their maximal weights were 104%, 93% and 51% greater than their presurgical weights, while the corresponding values for the control animals were 15% and 18% (Table 1). There was conspicuous accumulation of fat in the experimental animals.

The lesions extended 2.5–3.0 mm in the anteroposterior dimension. Their anterior extremes were no more than 0.5 mm posterior of the posterior border of the anterior commissure as it crosses the midline and the posterior extremes of the lesions were at the level of the anterior pole of the ventromedial hypothalamic nucleus (VMN). There was some variability in location of the lesions and in bilateral symmetry among animals, but the area common to all lesions was just inferior and lateral to the descending columns of the fornix (Fig. 1). The lesions extended laterally to the medial border of the internal capsule, interrupting ansa lenticularis at least unilaterally. The VMN was spared in 1720, but was slightly damaged unilaterally in 1572 and G67. The dorsal and ventral supraoptic commissures were damaged extensively.

The concentrations (ng/g wet weight) for the control animals of NE and DA for the brain regions that were sampled in this study were very similar to values obtained for larger groups of control animals maintained under similar conditions and evaluated according to the same assay [15]. Regional concentrations of catecholamines for the five animals in the present experiment are given in Table 1. The most striking effect of the lesions was a 59% decrease in NE in the cortical samples: the mean ($\pm$SD) of the pooled values for all oribital and dorsal cortical samples from each animal was 62 ± 7 ng/g for the experimentals vs 155 ± 4 ng/g for the controls. Every value for cortical NE concentration in the experimentals was below the range of the control values (Table 1). The experimentals also exhibited a 62% decrease in NE in the combined caudate and striatum samples (15 ± 11 ng/g vs 40 ± 6 ng/g). There was no consistent effect of the lesions on NE levels in the cerebellum or brain stem or on DA levels in any region sampled.

Although the experimentally obese animals had low concentrations of forebrain NE, there was not an obvious relationship within this group between NE levels and weight gain (Table 1). For example, the experimental animal that gained the least weight postlesion (51% above baseline) ranked highest in terms of NE depletion in the combined cortical samples (34% of control mean) and was intermediate in terms of NE depletion in the caudate-striatum samples (28% of control mean). The experimental animal that achieved the greatest weight gain (104% above baseline), on the other hand, had the smallest NE depletions in both the cortical samples (45% of control mean) and the caudate-striatum samples (75% of control mean).

DISCUSSION

Obesity in monkeys with lesions in this part of the hypothalamus has not been reported previously. The area common to all of the lesions in the present study was just inferior and lateral to the columns of the fornix. These lesions were more lateral than the effective lesions described by Hamilton to produce obesity [11] and more anterior than the lateral hypothalamic lesions which caused somnolence and weight loss in monkeys [2,22]. However, some parasagittal knife cuts which produce obesity in this species are included in the area where the lesions in the present study were located [6, 17, 20].

The obesity-related depletions of NE reported here are consistent with the report of obesity in stumptail macaques with NE depletions produced by lesions of LC [24,25], and it is very possible that the depletions of NE in the present study resulted from interruption of ascending projections from LC. This system has been shown to traverse the area of these hypothalamic lesions as a component of the medial forebrain bundle in *Macaca mulatta* as well as other species, including man, and to terminate in several forebrain regions including cerebral cortex [3, 7, 13, 21, 26]. Alternatively the cortical depletions may have resulted from destruction of cell bodies in the rostral hypothalamus whose axons terminate in frontal and parietal cortex [14]. The fact that DA levels in the regions studied were not significantly affected by these lesions is notable in light of work done in other species [29].

Despite the general correspondence between low cortical levels of NE and obesity in the experimental animals, the relationship may not be causal. Relative magnitudes of de-

pletions and weight gain were not clearly related within the experimental group. Also, lesions in the medial preoptic area (which would be expected to deplete cortical NE) did not produce obesity in this species [28], and intracerebroventricular administration of 6-hydroxydopamine has been shown to deplete cortical norepinephrine in monkeys without inducing obesity [15]. Rather, damage to hypothalamic systems, possibly including components of projections from LC, probably accounts for the obesity in these animals.

A model for adrenergic regulation of feeding behavior in the rat has been proposed in which hunger is mediated by α-adrenergic receptors and satiety by β-adrenergic receptors [18]. These hunger and satiety systems have been located for the rat in the medial and anterolateral hypothalamus, respectively [18]. There is some evidence that systems with similar neurochemical coding and neuroanatomical localization are present in the monkey [27]. Intrahypothalamic injection of NE can induce feeding in satiated monkeys and this effect is reduced by systemic pretreatment with phentolamine, an α-adrenergic antagonist. Similarly, phentolamine inhibits food intake in deprived monkeys. Pretreatment with β-adrenergic antagonists, on the other hand, either had no effect or increased food consumption. Furthermore, clonidine, an α-2 adrenergic agonist which may decrease function at α-1 and β receptors at low doses, has recently been shown to increase food intake in monkeys [26]. It seems reasonable therefore, to suggest that the obesity observed in the present study may have resulted from damage to a β-adrenergic satiety mechanism. Further research employing a variety of experimental approaches will be necessary to define the neurochemical and neuroanatomical systems for control of body weight and feeding behavior in primates.

REFERENCES

1. Ahlskog, J. E. and B. G. Hoebel. Overeating and obesity from damage to a noradrenergic system in the brain. *Science* **182:** 166–169, 1973.
2. Anand, B. K., S. Dua and K. Shoenberg. Hypothalamic control of food intake in cats and monkeys. *J. Physiol.* **127:** 143–152, 1955.
3. Bowden, D. M., D. C. German and W. D. Poynter. An autoradiographic, semistereotaxic mapping of major projections from locus coeruleus and adjacent nuclei in *Macaca mulatta*. *Brain Res.* **145:** 257–276, 1978.
4. Breese, G. R. and T. D. Traylor. Effect of 6-hydroxydopamine on brain norepinephrine and dopamine: Evidence for selective degeneration of catecholamine neurons. *J. Pharmac. exp. Ther.* **174:** 413–420, 1970.
5. Coscina, D. V., D. D. Godse and H. C. Stancer. Neurochemical correlates of hypothalamic obesity in rats. *Behav. Biol.* **16:** 365–372, 1976.
6. Ferin, M., J. L. Antunes, E. Zimmerman, I. Dyrenfurth, A. G. Frantz, A. Robinson and P. W. Carmel. Endocrine function in female rhesus monkeys after hypothalamic disconnection. *Endocrinology* **101:** 1611–1620, 1977.
7. German, D. C. and D. M. Bowden. Locus ceruleus in rhesus monkey (*Macaca mulatta*): A combined histochemical fluorescence, Nissl and silver study. *J. comp. Neurol.* **161:** 19–30, 1975.
8. Glick, S. D., S. Greenstein and D. H. Waters. Ventromedial hypothalamic lesions and brain catecholamines. *Pharmac. Biochem. Behav.* **1:** 591–592, 1973.
9. Grossman, S. P. The biology of motivation. *A. Rev. Psychol.* **30:** 209–242, 1979.
10. Grossman, S. P. and L. Grossman. Food and water intake in rats after transections of fibers *en passage* in the tegmentum. *Physiol. Behav.* **18:** 647–658, 1977.
11. Hamilton, C. L., P. J. Ciaccia and D. O. Lewis. Feeding behavior in monkeys with and without lesions of the hypothalamus. *Am. J. Physiol.* **230:** 818–830, 1976.
12. Hoebel, B. G. Pharmacologic control of feeding. *A. Rev. Pharmac. tox.* **17:** 605–621, 1977.
13. Hoffman, G. E., D. L. Felten and J. R. Sladek, Jr. Monoamine distribution in primate brain III. Catecholamine-containing varicosities in the hypothalamus of *Macaca mulatta*. *Am. J. Anat.* **147:** 501–514, 1976.
14. Kievet, J. and H. G. J. M. Kuypers. Subcortical afferents to the frontal lobe in the rhesus monkey studied by means of retrograde horseradish peroxidase transport. *Brain Res.* **85:** 261–266, 1975.
15. Kraemer, G. W., G. R. Breese, A. J. Prange, E. C. Moran, J. D. Lewis, J. W. Kemnitz, P. J. Bushnell, J. L. Howard and W. T. McKinney. Use of 6-hydroxydopamine to deplete brain catecholamines in the rhesus monkey: Effects on urinary catecholamine metabolites and behavior—A possible model of Parkinson's disease. *Psychopharmacology*, in press.
16. Kraemer, G. W., J. W. Kemnitz, W. T. McKinney and J. L. Howard. Use of pneumoencephalography to increase stereotaxic accuracy in rhesus monkeys. *Brain Res. Bull.* **3:** 155–160, 1978.
17. Krey, L. C., W. R. Butler and E. Knobil. Surgical disconnection of the medial basal hypothalamus and pituitary function in the rhesus monkey. I. Gonadotropin secretion. *Endocrinology* **96:** 1073–1087, 1975.
18. Leibowitz, S. F. Brain catecholaminergic mechanisms for control of hunger. In: *Hunger: Basic Mechanisms and Clinical Implications*, edited by D. Novin, W. Wyrwicka and G. Bray. New York: Raven Press, 1976, pp. 1–18.
19. Lorden, J., G. A. Oltmans and D. L. Margules. Central noradrenergic neurons: differential effects on body weight of electrolytic and 6-hydroxydopamine lesions in rats. *J. comp. physiol. Psychol.* **90:** 144–155, 1976.
20. McHugh, P. R. and J. Gibbs. Aspects of subcortical organization of feeding revealed by hypothalamic disconnexions in *Macaca mulatta*. *Brain* **95:** 279–292, 1972.
21. Nobin, A. and A. Björklund. Topography of the monoamine neuron systems in the human brain as revealed in fetuses. *Acta physiol. scand. suppl.* **388:** 1–40, 1973.
22. Ranson, S. W. Somnolence caused by hypothalamic lesions in the monkey. *Archs Neurol. Psychiat.* **41:** 1–23, 1939.
23. Ranson, S. W., C. Fisher and W. R. Ingram. Adiposity and diabetes mellitus in a monkey with hypothalamic lesions. *Endocrinology* **23:** 175–181, 1938.
24. Redmond, D. E., Jr., Y. H. Huang, J. Baulu, D. R. Snyder and J. W. Maas. Norepinephrine and satiety in monkeys. In: *Anorexia Nervosa*, edited by R. A. Vigersky. New York: Raven Press, 1977, pp. 81–96.
25. Redmond, D. E., Jr., Y. H. Huang, D. R. Snyder, J. W. Maas and J. Baulu. Hyperphagia and hyperdipsia after locus coeruleus lesions in the stump-tailed monkey. *Life Sci.* **20:** 1619–1628, 1977.
26. Schlemmer, F. R., Jr., R. C. Casper, N. Narasimhachari and J. M. Davis. Clonidine induced hyperphagia and weight gain in monkeys. *Psychopharmacology* **61:** 233–234, 1979.
27. Setler, P. E. and G. P. Smith. Increased food intake elicited by adrenergic stimulation of the diencephalon in rhesus monkeys. *Brain Res.* **65:** 459–473, 1974.
28. Slimp, J. C. Effects of medial preoptic-anterior hypothalamic lesions on heterosexual behavior, masturbation, and social behavior of male rhesus monkeys. Ph.D. Thesis, University of Wisconsin-Madison, 1976.
29. Ungerstedt, U. Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta physiol. scand. Suppl.* **367:** 1–48, 1971.