epigenetic mechanism could explain the high incidence of variants as well as our inability to detect any spontaneous revertants. The relative effectiveness of ICR-191 compared to ethylmethanesulfonate might also suggest an epigenetic event, since acridines can induce nongenetic changes, such as those causing petite mutants in yeast, at high rates (*18*). Harris (*19*) and Metzger-Freed (*20*) suggested that many of the variants arising from cultured cells may reflect changes in phenotypic expression of the sort that occurs during differentiation.

On the other hand, the variants described here could equally well be mutants arising from an alteration of chromosomal DNA. The incidence of light chain–producing and nonproducing variants is increased by nitrosoguanidine as well as by ICR-191. Most importantly, some of the variants induced by ICR-191 contain heavy chains that are abnormal in size and have apparent changes in their primary structure. A detailed structural analysis of the variant heavy chains may reveal the type of genetic mechanism responsible. For example, the ICR-191 variants producing small chains might be frame-shift mutants with a premature termination caused by a nonsense codon.

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Overeating and Obesity from Damage to a Noradrenergic System in the Brain

Abstract. *A discrete, ascending fiber system that supplies the hypothalamus with most of its noradrenergic terminals was destroyed at midbrain level, both electrolytically and with local injections of 6-hydroxydopamine, a destructive agent specific for catecholaminergic neurons. The result was hyperphagia leading to obesity. Fluorescence histochemical analysis showed that loss of noradrenergic terminals in ventral bundle termination areas such as the hypothalamus was necessary for hyperphagia. Damage to dorsal bundle or dopaminergic projections was not. Prior treatment with desmethylimipramine to selectively block uptake of 6-hydroxydopamine into noradrenergic neurons prevented both hyperphagia and loss of norepinephrine fluorescence. The lesions that produced hyperphagia also reduced the potency of* d-*amphetamine as an appetite suppressant. It is concluded that this noradrenergic bundle normally mediates suppression of feeding, thereby influences body weight, and serves as a substrate for* d-*amphetamine-induced loss of appetite.*

Recent studies have suggested a role for endogenous catecholamines in the inhibitory control of food intake. Pharmacological evidence suggests that amphetamines and related drugs that suppress food intake act by potentiation of adrenergic or dopaminergic transmission in the brain (*1*). Various experiments have pointed to the hypothalamus as the anatomical locus for at least part of this effect in rats (*2, 3*). Although dopaminergic terminals are virtually nonexistent in the hypothalamus, this region does contain high concentrations of noradrenergic terminals (*4*). Studies with fluorescence histochemistry have demonstrated that most or all hypothalamic noradrenergic terminals derive from long fibers ascending from hindbrain cell bodies (*5*); the majority of these fibers form a discrete bundle as they course through the ventrolateral tegmentum of the mesencephalon (ventral noradrenergic bundle) (*4, 6*). These facts suggested that the ventral noradrenergic bundle with its terminal plexus in the hypothalamus serves as a substrate for amphetamine-induced inhibition of feeding and, moreover, that it may function in normal regulation as a satiety system. If so, selective destruction of this nerve bundle should produce a satiety deficit, that is, disinhibited feeding. It should also render amphetamine less effective as an anoretic.

To determine if feeding is disinhibited, we destroyed the ventral noradrenergic bundle by two different methods in adult Sherman female rats. In one group this pathway was disrupted by discrete electrolytic lesions made at midbrain level (*7*). In another group 6-hydroxydopamine (6-OH-DA), which selectively destroys catecholaminergic neurons (*8, 9*), was injected directly into the ventral noradrenergic bundle (*10*). A third group received local 6-OH-DA injections into the ventral bundle at a more posterior midbrain locus (*11*). Control groups underwent treatment identical to the experimental groups but without passage of lesion current or addition of 6-OH-DA to the injection vehicle. Animals had free access to Purina laboratory pellets and water; food and water intake and body weight were measured every 48 hours.

All 43 animals given lesions or 6-OH-DA injections increased their food

intake after surgery (for example, Fig. 1). In approximately 80 percent of each 6-OH-DA group, food intake 40 days after surgery compared to that 16 days after surgery increased at least 25 percent; this criterion for hyperphagia was met by 58 percent of the group with electrolytic lesions (*12*). In several animals with lesions, overeating began within a few hours after the operation, and body weight gains of 10 to 25 g were not uncommon in the first 24 hours. Animals injected with 6-OH-DA generally displayed a 1- to 4-day latency in onset of the hyperphagia, as might be expected from the time course of action of this drug (*4, 9*). Weight decrements of 10 to 20 g often occurred in this group the first day after surgery, and hypophagia occasionally persisted for 2 to 3 days. However, in all groups given lesions or 6-OH-DA injections, once food consumption was elevated it did not return to baseline levels for the duration of the 40-day postoperative measurement period. In comparison, both sham surgery groups were unchanged (Table 1). Four months after the operation, a number of the hyperphagic animals were conspicuously obese, often at 150 percent of their estimated normal body weight.

Water consumption was elevated by only 16 percent in the experimental groups, compared to 4 percent in the control groups. In some cases drinking increments were transient even though hyperphagia was persistent. These hyperphagics did not overreact to taste adulteration of the diet (*13*).

If hyperphagia is the result of noradrenergic bundle destruction, then the overeating produced by injection of 6-OH-DA should be prevented by pharmacologically blocking the uptake of this drug into noradrenergic neurons. Desmethylimipramine (DMI) selectively blocks the uptake mechanism in noradrenergic but not dopaminergic systems (*14*); consequently, prior treatment with DMI has proved effective in selectively blocking 6-OH-DA–induced destruction of neurons containing norepinephrine but not of those containing dopamine (*15*). To determine if DMI could prevent the experimentally induced hyperphagia, 14 rats were injected with 6-OH-DA as in the first experiment (*10*), but half of these were first treated with DMI-HCl (50 mg per kilogram of body weight) injected intraperitoneally 30 to 75 minutes before 6-OH-DA administration. As a control for the effects of DMI alone, four animals were treated with DMI followed by sham surgery.

Table 1. Mean food consumption and body weight gain before and after surgery. The group with lesions underwent electrolytic destruction of the ventral noradrenergic bundle; the 6-OH-DA Ant. group was injected with 6-OH-DA at the level of the oculomotor nucleus; the 6-OH-DA Post. group was injected at the trochlear level. Postoperative body weight gain is given for a 30-day period because experimentally induced changes were maximum during this time.

Group	Food consumption (g/48 hours)		Change (%)	Body weight gain (g/48 hours)		Change (%)
	16 days before surgery	40 days after surgery		16 days before surgery	30 days after surgery	
Lesion ($N = 10$)	43.4	54.3	+ 25*	2.7	4.0	+ 48*
Sham ($N = 10$)	45.5	46.5	+ 2	3.1	2.0	− 35
6-OH-DA Ant. ($N = 14$)	42.3	56.6	+ 34*	3.3	4.7	+ 42*
6-OH-DA Post. ($N = 10$)	41.1	58.0	+ 41*	2.9	4.7	+ 62†
Sham ($N = 7$)	41.8	42.7	+ 2	3.0	1.7	− 43

* $P < .001$ for comparison with sham value (*16*). † $P < .005$ for comparison with sham value.

Treatment with DMI successfully blocked the development of hyperphagia in animals treated with 6-OH-DA. It did not affect food intake in the sham surgery group. In contrast, food consumption increased in the 6-OH-DA group not given DMI and was significantly different from that of the sham surgery group ($P < .001$) (*16*). (When consumption 8 days before and 30 days after surgery was compared, the mean increase was 1.5 percent for sham surgery controls, 0.8 percent for those given DMI and 6-OH-DA, and 44.9 percent for those given 6-OH-DA alone.) This supports the contention that it is destruction of a noradrenergic system which leads to hyperphagia.

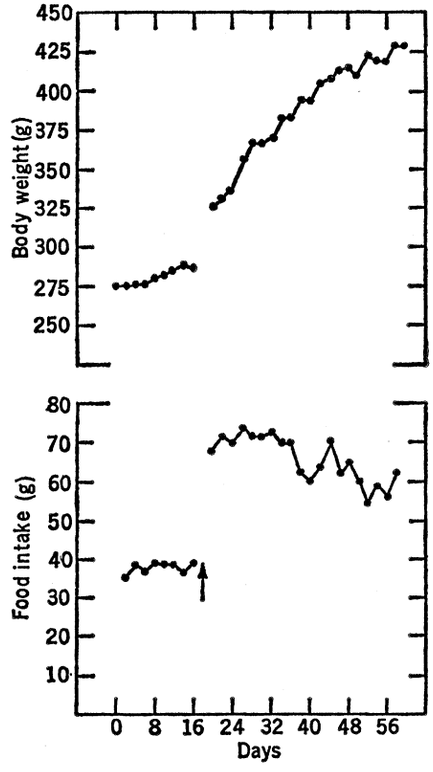

Fig. 1. Hyperphagia and weight gain of a representative animal before and after 6-OH-DA injection (arrow) into the ventral noradrenergic bundle.

The prevention of hyperphagia by DMI also argues against the possibility that unspecific destruction of noncatecholaminergic neurons mediates the increased consumption. Such nonselective damage should be independent of active uptake mechanisms; it should result instead from extraneuronal oxidation or after passive diffusion into cells. Thus, DMI should not affect this type of destruction. Consistent with this notion, areas of unspecific tissue loss and phagocytic activity were identical in the DMI-treated and -untreated animals given 6-OH-DA.

If the hyperphagia that we observed is the result of ventral and not dorsal noradrenergic destruction, then injection of 6-OH-DA into the dorsal bundle should prove ineffective. We attempted to selectively destroy this pathway at the point where it diverges dorsally from the ventral bundle by stereotaxically aimed 6-OH-DA injections in seven animals (*17*). Four control rats received injection of the vehicle only, and seven others were injected in the ventral bundle as in the first experiment (*10*). Only animals in the ventral bundle group became hyperphagic, and only this group was significantly different from the controls ($P < .05$). The mean increase in food intake was 2.3 percent for the control group, 1.5 percent for the dorsal bundle group, and 32.9 percent for the ventral bundle group. This supports the contention that it is destruction of the ventral, not dorsal, noradrenergic system which leads to hyperphagia.

Brain catecholamine depletion was analyzed by the Falck-Hillarp fluo-

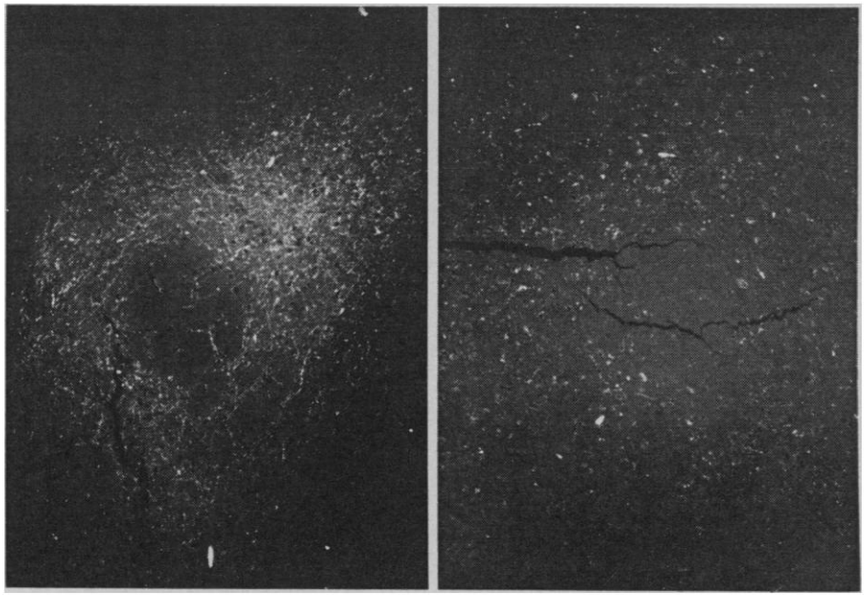

Fig. 2. Fluorescence photomicrographs of perifornical region of the hypothalamus. (Left) Abundant catecholamine varicosities envelop fornix of sham operated rat. (Right) Nearly total depletion of catecholamine fluorescence is seen in the same region of a hyperphagic rat that had been injected with 6-OH-DA in the vicinity of the ventral noradrenergic bundle. Bright particles remaining are yellow autofluorescent lipofuscin granules.

rescence histochemical method (*18*). Catecholamine fluorescence in seven representative hyperphagic rats injected with 6-OH-DA in the ventral bundle was evaluated relative to sham injected animals. In six of the seven there was almost complete loss of fluorescent varicosities in the hypothalamic and forebrain areas known to be innervated by noradrenergic fibers; this indicated that all noradrenergic input, dorsal and ventral, had been destroyed (Fig. 2). In the seventh hyperphagic rat, a large but less complete depletion was noted. In contrast, four representative 6-OH-DA animals first treated with DMI appeared to have normal numbers of fluorescent varicosities in all noradrenergic areas; these animals were not hyperphagic.

With respect to the striatum and other dopaminergic projection areas, the catecholamine fluorescence appeared normal or nearly normal in six of seven rats injected with 6-OH-DA in the ventral bundle without prior DMI treatment; the pattern and intensity of fluorescence in these regions was identical to that of the 6-OH-DA group first treated with DMI. In summary, histochemical examination confirmed that hyperphagia probably resulted from loss of norepinephrine.

Further histochemical evidence from separate destruction of the dorsal and ventral bundles pointed specifically to ventral bundle damage as the cause of the hyperphagia syndrome. Analysis of three animals injected with 6-OH-DA in the dorsal bundle revealed an almost complete loss of catecholamine varicosities in the dorsal bundle projection areas (cortex, hippocampus, and most of the thalamus) but only a small reduction in those of the ventral bundle, such as the hypothalamus; these animals did not become hyperphagic. Furthermore, three additional rats with electrolytic lesions in the ventral bundle displayed normal noradrenergic innervation of the cortex and thalamus but had a large reduction of catecholaminergic terminals in all ventral bundle projection areas; these animals did become hyperphagic. Thus, only when the ventral noradrenergic bundle was destroyed did overeating occur (*19*).

Assay of forebrain monoamines (*20*) in eight hyperphagic rats injected with 6-OH-DA (*10*) and eight sham injected rats yielded results consistent with the fluorescence histochemistry. In the hyperphagic rats, norepinephrine was reduced to 15 percent of the control mean or less, dopamine was 84 to 93 percent, and serotonin was 97 to 106 percent.

Since our evidence suggests that the ventral bundle mediates satiety, it follows that its destruction might attenuate *d*-amphetamine-induced satiety. To investigate this possibility, nine rats with electrolytic lesions of the ventral noradrenergic bundle (*21*) and seven sham operated animals were periodically injected intraperitoneally with *d*-amphetamine sulfate ½ hour before their daily 4-hour feeding period. Half of each group received five *d*-amphetamine doses in ascending order (0.5, 1.0, 2.0, 4.0, and 8.0 mg/kg); the other half received these doses in the reverse order. The amount eaten in the first hour after amphetamine injection was compared with that from the previous day, when saline had been injected.

The results were in accord with our hypothesis. Destruction of the ventral noradrenergic bundle diminished the anorectic action of all but the highest dose of *d*-amphetamine. For example, rats with lesions still ate 92 percent of their normal food intake after a *d*-amphetamine dose of 0.5 mg/kg, whereas intake in sham operated rats was only 53 percent of normal ($P < .02$). In effect, the dose-response curve was shifted to the right. This suggests that, in intact animals, amphetamines act in part by potentiating synaptic transmission at terminals of ventral noradrenergic bundle neurons.

Norepinephrine cannulated into the hypothalamus elicits eating. Early workers concluded that endogenous norepinephrine does the same thing (*22*). It has also been postulated that lateral hypothalamic damage produces a deficit in noradrenergic function and reduces feeding (*23*). Our results are in marked contrast to the idea that synaptic release of endogenous norepinephrine elicits feeding or that norepinephrine loss produces a feeding decrement. Although noradrenergic transmission may be sufficient to facilitate feeding (*3, 22–24*), it does not appear to be necessary. To the contrary, destruction of the major afferent norepinephrine pathway to the hypothalamus by itself or in conjunction with the other ascending noradrenergic systems does not result in anorexia, but instead leads to hyperphagia (*25*). This is consistent with previous evidence for noradrenergic receptors mediating satiety (*26*). In summary, the results suggest that (i) norepinephrine is a neurotransmitter in a satiety mechanism; (ii) the specific anatomical pathway involved is the ventral noradrenergic bundle; and (iii) this system may serve as a substrate for amphetamine-induced anorexia.

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10. A single dose of 8.0 μg of 6-OH-DA hydrochloride dissolved in 0.8 μl of saline (containing ascorbic acid, 0.2 μg/μl) was bilaterally injected at 0.4 μl/min through a stereotaxically lowered 27- or 32-gauge cannula, which was withdrawn 4 to 5 minutes after completion of the injection; coordinates were as in (*7*). Placement was verified in all rats.
11. Coordinates: A0.2 (level of the trochlear nucleus), L1.5, D6.9; same procedure as in (*10*).
12. Five of six additional rats treated with more dilute 6-OH-DA (8 μg/4 μl) also became hyperphagic; the mean increase for all six was 38.8 percent.
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19. In a more recent series of rats, one animal markedly depleted of noradrenergic fluorescent varicosities in the diencephalon and telencephalon failed to become hyperphagic. Evidently, norepinephrine depletion is necessary for the hyperphagia syndrome reported here but may not always be sufficient for it. T. Maedo and H. Shimizu [*Brain Res.* **36**, 19 (1972)] described a second, smaller noradrenergic projection to the hypothalamus in addition to the ventral bundle. Since our histological examination confirmed that electrolytic lesions leading to hyperphagia spared the region through which this bundle projects, we conclude that its destruction is not necessary for feeding increases. However, whether damage to this pathway contributes to the hyperphagia is undetermined.
20. Assays for all three neurotransmitters were performed on homogenate fractions of the same brains by a slight modification of the techniques of C. L. Chang [*Int. J. Neuropharmacol.* **3**, 643 (1964)]; R. P. Maickel, R. H. Cox, J. Saillant, F. P. Miller [*ibid.* **7**, 275 (1968)]; and D. R. Haubrich and J. S. Penzer (*Anal. Biochem.*, in press).
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25. Since the ventral noradrenergic bundle also projects to extrahypothalamic areas, the present results may reflect norepinephrine loss in these regions as well as in the hypothalamus.
26. An α-adrenergic (norepinephrine-elicited) satiety mechanism has been proposed by D. L. Margules [*Life Sci.* **8**, 693 (1969); *J. Comp. Physiol. Psychol.* **73**, 1 (1970)]. It is not clear how our findings relate to theories of β-adrenergic (isoproterenol-elicited) satiety [D. L. Margules, *J. Comp. Physiol. Psychol.* **73**, 1 (1970); S. F. Leibowitz (*3*); H. W. Goldman, D. Lehr, E. Friedman, *Nature* **231**, 453 (1971)] unless mediated by norepinephrine [S. F. Leibowitz, *Proceedings, 79th Annual Convention of the APA* (American Psychological Association, Washington, D.C., 1971), p. 741].
27. An abstract of some of this work has appeared (*13*). Later experiments are part of the dissertation of J.E.A. submitted to the department of psychology, Princeton University. We thank P. K. Randall for performing the monoamine assays.

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Amphibian Pituitary Growth Hormone and Prolactin: Immunochemical Relatedness to Rat Growth Hormone

Abstract. *Growth hormone and prolactin were electrophoretically isolated from amphibian pituitaries and then were tested in a radioimmunoassay with labeled rat growth hormone and antiserum to the same hormone. This isolation and purification of the hormones increased the steepness of the slopes of competitive inhibition in this system when compared to those of crude extracts. Both hormones from most species tested showed high immunochemical cross-reactivity, indicating that amphibian growth hormone and prolactin are structurally related to rat growth hormone.*

The results of comparative immunochemical studies indicated that the growth hormones (GH's) in extracts of pituitaries of nonmammalian tetrapods fall into major immunochemical categories that correspond closely to their phylogenetic relationship to mammals (*1*). Thus there may be common immunochemical molecular features (determinants) among the GH's of all tetrapods, and the number of such common determinants may vary with phylogeny. However, there are several problems in interpreting the results of such studies when they are based on the use of crude pituitary extracts. In particular, we must consider the possibility that the immunological reactivity in the pituitary extracts from certain nonmammalian species may not be due solely to GH.

Comparative bioassays of GH's and prolactins (PRL's) have revealed a significant overlap in the biological properties of these two hormones from diverse tetrapod species (*2*); the occurrence of such overlap is not predictable on the basis of phylogenetic relationships. In addition, the primary structures of these two hormones from mammalian pituitaries are similar (*3*). Either or both hormones may possess common immunologic determinants in some species but the occurrence of these common determinants may be unrelated to phylogeny. We now present evidence that such phylogenetically unrelated cross-reactivities occur among PRL's and GH's which were isolated from pituitaries of diverse amphibian species.

We separated GH and PRL from fresh frozen pituitary glands of several anuran and urodele amphibia by polyacrylamide disc electrophoresis (*2*). The two hormones are well separated by this procedure; the PRL's have a high electrophoretic mobility relative to the GH's of all species (*2*, *4*). The protein bands containing these hormonal activities from extracts of adenohypophyses from several amphibian species were previously identified by bioassays. We used the linear growth test in juvenile toads to identify GH (*2*) and the local pigeon crop sac for PRL (*4*).

In the present study, the hormones were obtained by electrophoretic separation of extracts of pooled glands from adults of both sexes (Table 1). We tested the efficacy of the electrophoretic system for the separation of the hormones by using rat adenohypophyses which were processed similarly to the amphibian adenohypophyses.